PCT WORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

B2

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 7 : C12Q 1/68, C12N 5/10

A2

(11) International Publication Number: WO 00/70099

(43) International Publication Date: 23 November 2000 (23.11.00)

(21) International Application Number: PCT/US00/13951

(22) International Filing Date: 19 May 2000 (19.05.00)

(30) Priority Data:

60/134,790	19 May 1999 (19.05.99)	US
09/574,613	18 May 2000 (18.05.00)	US

(71) Applicant *(for all designated States except US)*: MITOKOR [US/US]; 11494 Sorrento Valley Road, San Diego, CA 92121 (US).

(72) Inventors; and

(75) Inventors/Applicants *(for US only)*: HWANG, Jung-Joo [KR/US]; #4, 3773 Carmel View Road, San Diego, CA 92130 (US). BECKER, K., David [US/US]; 12056 Mannix Road, San Diego, CA 92129 (US). DAVIS, Robert, E. [US/US]; 13272 Glencliff Way, San Diego, CA 92130 (US).

(74) Agents: ROSENMAN, Stephen, J. et al.; Seed Intellectual Property Law Group PLLC, Suite 6300, 701 Fifth Avenue, Seattle, WA 98104-7092 (US).

(81) Designated States: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW, ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).

Published

Without international search report and to be republished upon receipt of that report.

(54) Title: DIFFERENTIAL GENE EXPRESSION IN SPECIFIC REGIONS OF THE BRAIN IN NEURODEGENERATIVE DISEASES

(57) Abstract

The present invention provides nucleic acid molecules that can function as PCR primers for the detection of contactin mRNA in a sample. Such nucleic acid molecules can be labeled and can be provided in a kit. The present invention also includes a method of detecting the presence of a neurodegenerative disease such as multiple sclerosis, including providing a sample from a patient and measuring the amount of contactin protein or contactin mRNA expressed in the sample. The present invention further provides a method for identifying which patents having a neurodegenerative disease are likely to respond to a treatment for a neurodegenerative disease. The present invention further includes compositions of matter that include an isolated cell or a cell in culture that expresses an increased or decreased amount of contactin as compared to a control cell. The present invention also includes a method for screening compounds for the activity of reducing or enhancing the expression of contactin and compositions or compounds, including pharmaceutical compositions, identified by this method. Also included are methods of treating a neurodegenerative disease using such compositions or compounds. The present invention also includes methods of identifying pharmaceutical targets for compounds that modulate contactin expression or activity and targets identified by such methods.

FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AL	Albania
AM	Armenia
AT	Austria
AU	Australia
AZ	Azerbaijan
BA	Bosnia and Herzegovina
BB	Barbados
BE	Belgium
BF	Burkina Faso
BG	Bulgaria
BJ	Benin
BR	Brazil
BY	Belarus
CA	Canada
CF	Central African Republic
CG	Congo
CH	Switzerland
CI	Côte d'Ivoire
CM	Cameroon
CN	China
CU	Cuba
CZ	Czech Republic
DE	Germany
DK	Denmark
EE	Estonia
ES	Spain
FI	Finland
FR	France
GA	Gabon
GB	United Kingdom
GE	Georgia
GH	Ghana
GN	Guinea
GR	Greece
HU	Hungary
IE	Ireland
IL	Israel
IS	Iceland
IT	Italy
JP	Japan
KE	Kenya
KG	Kyrgyzstan
KP	Democratic People's Republic of Korea
KR	Republic of Korea
KZ	Kazakstan
LC	Saint Lucia
LI	Liechtenstein
LK	Sri Lanka
LR	Liberia
LS	Lesotho
LT	Lithuania
LU	Luxembourg
LV	Latvia
MC	Monaco
MD	Republic of Moldova
MG	Madagascar
MK	The former Yugoslav Republic of Macedonia
ML	Mali
MN	Mongolia
MR	Mauritania
MW	Malawi
MX	Mexico
NE	Niger
NL	Netherlands
NO	Norway
NZ	New Zealand
PL	Poland
PT	Portugal
RO	Romania
RU	Russian Federation
SD	Sudan
SE	Sweden
SG	Singapore
SI	Slovenia
SK	Slovakia
SN	Senegal
SZ	Swaziland
TD	Chad
TG	Togo
TJ	Tajikistan
TM	Turkmenistan
TR	Turkey
TT	Trinidad and Tobago
UA	Ukraine
UG	Uganda
US	United States of America
UZ	Uzbekistan
VN	Viet Nam
YU	Yugoslavia
ZW	Zimbabwe

DIFFERENTIAL GENE EXPRESSION IN SPECIFIC REGIONS OF THE BRAIN IN NEURODEGENERATIVE DISEASES

FIELD OF THE INVENTION

The present invention generally relates generally to the fields of molecular biology, neurobiology, neurodegenerative diseases and the diagnosis and treatment thereof.

BACKGROUND OF THE INVENTION

Neurodegenerative diseases afflict humans with a variety of debilitating effects, such as memory loss, loss of musculoskeletal and fine motor control or coma. It has been recognized that some neurodegenerative diseases, such as Alzheimer's disease (AD), Parkinson's disease (PD), diffuse Lewy body disease (DLB), vascular dementia, multiple sclerosis (MS), amyotrophic lateral sclerosis (ALS) myoclonic epilepsy lactic acidosis and stroke (MELAS) and myoclonic epilepsy ragged red fiber syndrome (MERRF) are or may be related to mitochondrial defects.

Parkinson's disease (PD) is a progressive, neurodegenerative disorder associated with altered mitochondrial function and characterized by the loss and/or atrophy of dopamine-containing neurons in the *pars compacta* of the *substantia nigra* of the brain. Like Alzheimer's Disease (AD), PD also afflicts the elderly. It is characterized by bradykinesia (slow movement), rigidity and a resting tremor. Although L-Dopa treatment reduces tremors in most patients for a while, ultimately the tremors become more and more uncontrollable, making it difficult or impossible for patients to even feed themselves or meet their own basic hygiene needs.

It has been shown that the neurotoxin 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) induces parkinsonism in animals and man at least in part through its effects on mitochondria. MPTP is converted to its active metabolite, MPP+, in dopamine neurons; it then becomes concentrated in the mitochondria. The MPP+ then selectively inhibits the mitochondrial enzyme NADH:ubiquinone oxidoreductase

("Complex I"), leading to the increased production of free radicals, reduced production of adenosine triphosphate, and ultimately, the death of affected dopamine neurons.

Mitochondrial Complex I is composed of 40-50 subunits; most are encoded by the nuclear genome and seven by the mitochondrial genome. Since parkinsonism may be induced by exposure to mitochondrial toxins that affect Complex I activity, it appears likely that defects in Complex I proteins may contribute to the pathogenesis of PD by causing a similar biochemical deficiency in Complex I activity. Indeed, defects in mitochondrial Complex I activity have been reported in the blood and brain of PD patients (Parker et al., *Am. J. Neurol. 26*:719-723, 1989).

Alzheimer's disease (AD) is a progressive neurodegenerative disorder that is characterized by loss and/or atrophy of neurons in discrete regions of the brain, and that is accompanied by extracellular deposits of β-amyloid and the intracellular accumulation of neurofibrillary tangles. It is a uniquely human disease, affecting over 13 million people worldwide. It is also a uniquely tragic disease. Many individuals who have lived normal, productive lives are slowly stricken with AD as they grow older, and the disease gradually robs them of their memory and other mental faculties. Eventually, they cease to recognize family and loved ones, and they often require continuous care until their eventual death.

There is evidence that defects in oxidative phosphorylation within the mitochondria are at least a partial cause of sporadic AD. The enzyme cytochrome c oxidase (COX), which makes up part of the mitochondrial electron transport chain (ETC), is present in normal amounts in AD patients; however, the catalytic activity of this enzyme in AD patients and in the brains of AD patients at autopsy has been found to be abnormally low. This suggests that the COX in AD patients is defective, leading to decreased catalytic activity that in some fashion causes or contributes to the symptoms that are characteristic of AD.

Focal defects in energy metabolism in the mitochondria, with accompanying increases in oxidative stress, may be associated with AD. It is well-established that energy metabolism is impaired in AD brain (Palmer et al., *Brain Res.*

645:338-42, 1994; Pappolla et al., *Am. J. Pathol. 140*:621-28, 1992; Jeandel et al., *Gerontol. 35*:275, 1989; Balazs et al., *Neurochem. Res. 19*:1131-37, 1994; Mecocci et al., *Ann. Neurol. 36*:747-751, 1994; Gsell et al., *J. Neurochem. 64*:1216-23, 1995). For example, regionally specific deficits in energy metabolism in AD brains have been reported in a number of positron emission tomography studies (Kuhl, et al., *J. Cereb. Blood Flow Metab.* 7:S406, 1987; Grady, et al., *J. Clin. Exp. Neuropsychol. 10*:576-96, 1988; Haxby et al., *Arch. Neurol. 47*:753-60, 1990; Azari et al., *J. Cereb. Blood Flow Metab. 13*:438-47, 1993). Metabolic defects in the temporoparietal neocortex of AD patients apparently presage cognitive decline by several years. Skin fibroblasts from AD patients display decreased glucose utilization and increased oxidation of glucose, leading to the formation of glycosylation end products (Yan et al., *Proc. Nat. Acad. Sci. USA 91*:7787-91, 1994). Cortical tissue from postmortem AD brain shows decreased activity of the mitochondrial enzymes pyruvate dehydrogenase (Sheu et al., *Ann. Neurol. 17*:444-49, 1985) and α–ketoglutarate dehydrogenase (Mastrogiacomo et al., *J. Neurochem. 6*:2007-14, 1994), which are both key enzymes in energy metabolism. Functional magnetic resonance spectroscopy studies have shown increased levels of inorganic phosphate relative to phosphocreatine in AD brain, suggesting an accumulation of precursors that arises from decreased ATP production by mitochondria (Pettegrew et al., *Neurobiol. of Aging 15*:117-32, 1994; Pettigrew et al., *Neurobiol. of Aging 16*:973-75, 1995). In addition, the levels of pyruvate, but not of glucose or lactate, are reported to be increased in the cerebrospinal fluid of AD patients, consistent with defects in cerebral mitochondrial electron transport chain (ETC) activity (Parnetti et al., *Neurosci. Lett. 199*:231-33, 1995).

Signs of oxidative injury are prominent features of AD pathology and, as noted above, reactive oxygen species (ROS) are critical mediators of neuronal degeneration. Indeed, studies at autopsy show that markers of protein, DNA and lipid peroxidation are increased in AD brain (Palmer et al., *Brain Res.* 645:338-42, 1994; Pappolla et al., *Am. J. Pathol.* 140:621-28, 1992; Jeandel et al., *Gerontol.* 35:275-82, 1989; Balazs et al., *Arch. Neurol.* 4:864, 1994; Mecocci et al., *Ann. Neurol.* 36:747-51,

1994; Smith et al., *Proc. Nat. Acad. Sci. USA* 88:10540-43, 1991). In hippocampal tissue from AD but not from controls, carbonyl formation indicative of protein oxidation is increased in neuronal cytoplasm, and nuclei of neurons and glia (Smith et al., *Nature 382*:120-21, 1996). Neurofibrillary tangles also appear to be prominent sites of protein oxidation (Schweers et al., *Proc. Nat. Acad. Sci. USA 92*:8463, 1995; Blass et al., *Arch. Neurol. 4*:864, 1990). Under stressed and non-stressed conditions incubation of cortical tissue from AD brains taken at autopsy demonstrate increased free radical production relative to non-AD controls. In addition, the activities of critical antioxidant enzymes, particularly catalase, are reduced in AD (Gsell et al., *J. Neurochem. 64*:1216-23, 1995), suggesting that the AD brain is vulnerable to increased ROS production. Thus, oxidative stress may contribute significantly to the pathology of mitochondria associated diseases such as AD, where mitochondrial dysfunction and/or elevated ROS may be present.

One hallmark pathology of AD is the death of selected neuronal populations in discrete regions of the brain. Cell death in AD is presumed to be apoptotic because signs of programmed cell death (PCD) are seen and indicators of active gliosis and necrosis are not found. (Smale et al., *Exp. Neurolog. 133*:225-230, 1995; Cotman et al., *Molec. Neurobiol. 10*:19-45, 1995.) The consequences of cell death in AD, neuronal and synaptic loss, are closely associated with the clinical diagnosis of AD and are highly correlated with the degree of dementia in AD (DeKosky et al., *Ann. Neurology 27*:457-464, 1990).

Mitochondrial dysfunction is thought to be critical in the cascade of events leading to apoptosis in various cell types (Kroemer et al., *FASEB J. 9*:1277-87, 1995), and may be a cause of apoptotic cell death in neurons of the AD brain. Altered mitochondrial physiology may be among the earliest events in PCD (Zamzami et al., *J. Exp. Med. 182*:367-77, 1995; Zamzami et al., *J. Exp. Med. 181*:1661-72, 1995) and elevated reactive oxygen species (ROS) levels that result from such altered mitochondrial function may initiate the apoptotic cascade (Ausserer et al., *Mol. Cell. Biol. 14*:5032-42, 1994). In several cell types, including neurons, reduction in the

mitochondrial membrane potential ($\Delta\Psi m$) precedes the nuclear DNA degradation that accompanies apoptosis. In cell-free systems, mitochondrial, but not nuclear, enriched fractions are capable of inducing nuclear apoptosis (Newmeyer et al., *Cell 70*:353-64, 1994). Perturbation of mitochondrial respiratory activity leading to altered cellular metabolic states, such as elevated intracellular ROS, may occur in mitochondria associated diseases and may further induce pathogenetic events via apoptotic mechanisms.

Oxidatively stressed mitochondria may release a pre-formed soluble factor that can induce chromosomal condensation, an event preceding apoptosis (Marchetti et al., *Cancer Res. 56*:2033-38, 1996). In addition, members of the Bcl-2 family of anti-apoptosis gene products are located within the outer mitochondrial membrane (Monaghan et al., *J. Histochem. Cytochem. 40*:1819-25, 1992) and these proteins appear to protect membranes from oxidative stress (Korsmeyer et al, *Biochim. Biophys. Act. 1271*:63, 1995). Localization of Bcl-2 to this membrane appears to be indispensable for modulation of apoptosis (Nguyen et al., *J. Biol. Chem. 269*:16521-24, 1994). Thus, changes in mitochondrial physiology may be important mediators of apoptosis. To the extent that apoptotic cell death is a prominent feature of neuronal loss in AD, mitochondrial dysfunction may be critical to the progression of this disease and may also be a contributing factor in other mitochondria associated diseases.

Regardless of whether a defect underlying a disease associated with altered mitochondrial function may have mitochondrial or extramitochondrial origins, and regardless of whether a defect underlying altered mitochondrial function has been identified, the present invention provides methods that are useful for determining the risk or presence of diseases associated with such altered mitochondrial function, and for identifying agents that are suitable for treating such diseases.

Diffuse Lewy body disease (DLB), or Lewy body dementia, is a degenerative disorder of the central nervous system (CNS) that typically presents in older patients, initially as psychosis or progressively deteriorating dementia that may precede tremors, rigidity of movement or other manifestations of parkinsonism.

Pathology of DLB reveals diffuse distributions of intracytoplasmic neuronal inclusions known as Lewy bodies, particularly in the nuclei of brainstem, basal forebrain and hypothalamic neurons. DLB may also be accompanied by one or more of myoclonus, dysphagia, orthostatic hypotension and involuntary movements of the skeletal muscles.

Vascular dementia, or "multi-infarct", refers to a variety of disorders characterized by progressively deteriorating cognitive abilities that results from multiple infarction events in the cerebral vasculature. Impaired memory and intellectual ability are typically accompanied by focal neurologic signs in vascular dementia.

Multiple sclerosis is a chronic disease in humans that is related to central nervous system demyelination, occurring either as a succession of phases characterized by alternating remission and exacerbation ("relapsing/remitting" disease), or as a steadily progressing disease, often leading to paralysis. The anatomopathological features of this disease include well-delimited patches of demyelination in the white matter of the brain and spinal cord. It is believed that inflammatory, genetic, environmental and etiological factors contribute to the pathogenesis of multiple sclerosis.

Amyotrophic lateral sclerosis (ALS) is a commonly diagnosed progressive motor neuron disease. The disease is characterized by degeneration of motor neurons in the cortex, brainstem and spinal cord. The onset of the disease is between about the third and sixth decade and is uniformly fatal. The cause of ALS is unknown, and is diagnosed when symptoms, such as asymmetric limb weakness, localized fasciculation of the limbs or spasticity in the legs are noted.

Myoclonic epilepsy lactic acidosis and stroke (MELAS) is a disease characterized by stroke-like episodes and lactic acidosis. The stroke-like episodes can be precipitated by metabolic stress. The disease results in neurological pathologies including multiple infarction-like lesions with various degrees of generalized cerebral and cerebellar atrophy that are not related to the vascular territories of the central nervous system.

Myoclonic epilepsy ragged red fiber syndrome (MERRF) is characterized by sensor and motor skill dysfunction, lactic acidosis, encephalopathy, stroke-like episodes, seizures and muscle weakness. The disease results in microscopic degeneration of central nervous system tissues with loss of nerve cells.

Human contactin protein, and its homologs from mouse (F3 protein) and chicken (F11 protein), are cell surface adhesion proteins that are involved in cell attachment to substrate. Contactin comprises an Ig-like domain and multiple fibronectin III-like domains (Brummendorf et al., J. Neurochemistry 61:1207-1219 (1993)). Unlike many cell adhesion molecules, contactin is not a transmembrane protein, but instead is anchored to cell surfaces via linkage to glycosylphosphatidylinositol (GPI) in the plasma membrane outer leaflet (*Id.*). In human tissues, relatively high levels of a major contactin mRNA (6.5 kb) are expressed in adult brain along with three minor transcripts (9.7 kb, 4.4 kb and 3.4 kb), whereas low levels of expression of multiple forms of contactin mRNA are found in the adult lung, pancreas, kidney and skeletal muscles (6.8 kb and 6.0 kb) (Reid et al., Molecular Brain Research 21:1-8 (1994)). High levels of expression of the multiple forms of contactin mRNA are found in neuroblastoma and retinoblastoma cell lines (6.8 kb, 6.0 kb and 4.2 kb) (Id.). The expression of contactin in developing neural tissue is complex, transient, and temporally regulated. Contactin is believed to have a role in neurite outgrowth, perhaps by binding to the cell recognition molecule Ng-CAM and/or by interacting with the extracellular matrix glycoprotein restrictin (Faivre-Sarrailh et al., J. Neurosci. 12:257-267 (1992), Brummendorf et al., Neuron 10:711-7272 (1993)). Adult neural stem cells can give rise to hematopoietic cells, including cells of the myeloid and lymphoid lineages (Bjornson et al., Science 283:534-537 (1999)); thus, contactin mRNA, contactin DNA or contactin protein may be detected in blood.

Identification of the underlying causes of neurodegenerative diseases has often been elusive, as has been the development of reliable diagnosis, prognosis and treatment of such diseases. There clearly exists a need to provide improved methods and compositions for treating these neurodegenerative diseases. The present invention

satisfies these needs by providing the first recognition of a role for contactin in neurodegenerative diseases such as these described above, and further provides other related advantages.

SUMMARY OF THE INVENTION

The present invention is directed in part to exploitation of the relationship between altered expression levels (*e.g.*, increases or decreases in a statistically significant manner) of certain nucleic acid molecules in cells to neurodegenerative diseases, where the nucleic acid molecules that exhibit altered expression levels encode particular products, including contactin. FREAC-2, APCL, LAP, COX7C, PAF, 6PTS1, VDAC1, UNK-Br40, UNK-Br42, a novel brain-expressed EFHD homologue and a variety of additional novel products, as well as a number of known products as described herein. The present disclosure is therefore directed in pertinent part to a plurality of such nucleic acid molecules having expression levels that are altered in one or more cell type or tissue in at least one neurodegenerative disease, where nucleic acid molecules that encode contactin comprise but one representative example of the nucleic molecules having altered expression levels that correlate with neurodegenerative disease. Accordingly, although abundant reference is made herein to altered expression levels of nucleic acid molecules that encode contactin as such a representative example, it is to be understood that the present invention relates as well to the several additional nucleic acid molecules described herein that encode other products, including products having novel sequences and products having known sequences.

Thus more specifically, according to the present invention there is provided a correlation between cellular expression levels of the cell adhesion molecule contactin and the presence or risk of a neurodegenerative disease, such that quantitative alterations or modulation of cellular contactin expression (*e.g.*, increases or decreases) relate to neurodegenerative diseases, relative to situations where such diseases are absent.

In one aspect, the invention provides an oligonucleotide primer capable of specifically amplifying DNA or RNA encoding contactin or a nucleic acid sequence complementary thereto, comprising an isolated nucleic acid molecule that is identical or substantially identical to a nucleotide sequence that is SEQ ID NO:1 or a portion thereof, SEQ ID NO:2 or a portion thereof, SEQ ID NO:3 or a portion thereof, SEQ ID NO:4 or a portion thereof, SEQ ID NO:5 or a portion thereof, SEQ ID NO:6 or a portion thereof or SEQ ID NO:7 or a portion thereof. In certain embodiments, the isolated nucleic acid molecule comprises a detectable label. The invention also provides a kit comprising an oligonucleotide primer as just described.

It is another aspect of the invention to provide a method of detecting the risk for having or presence of a neurodegenerative disease in a subject, comprising comparing the amount of contactin protein in a test sample from a subject to the amount of contactin protein in a sample from a control subject known to be free of a risk for having or presence of the neurodegenerative disease. The invention also provides, in certain embodiments, a method of detecting the risk for having or presence of a neurodegenerative disease in a subject, comprising comparing the amount of contactin mRNA in a test sample from a subject to the amount of contactin mRNA in a sample from a control subject known to be free of a risk for having or presence of the neurodegenerative disease. In certain embodiments, the test sample and the control sample are derived from central nervous system. In certain other embodiments, contactin mRNA is measured by a polymerase chain reaction method. In certain further embodiments, the polymerase chain reaction method comprises amplification with a forward primer comprising a nucleotide sequence of SEQ ID NO:1, SEQ ID NO:3, SEQ. ID NO:5 or SEQ ID NO:6. In certain other embodiments, the polymerase chain reaction method comprises amplification with a reverse primer comprising a nucleotide sequence of SEQ ID NO:2, SEQ ID NO:4, or SEQ ID NO: 7.

In certain embodiments, the invention provides a method of detecting the risk for having or presence of a neurodegenerative disease in a subject, comprising comparing the amount of contactin mRNA in a test sample from a subject to the amount

of RNA in a sample from a control subject known to be free of a risk for having or presence of the neurodegenerative disease, wherein the control sample RNA does not encode contactin. In certain embodiments, the neurodegenerative disease is amyotrophic lateral sclerosis, multiple sclerosis, MELAS or MERRF, and in certain preferred embodiments the the neurodegenerative disease is multiple sclerosis.

The present invention also provides, in another aspect, a method of screening an agent for use in treating patients having a neurodegenerative disease, comprising determining a first level of contactin expression in a first sample from at least one patient prior to contacting the patient with a candidate agent, wherein the step of determining is determining an amount of contactin protein or determining an amount of contactin RNA; and comparing the first level of contactin expression to a second level of contactin expression determined in a second sample from the patient after contacting the patient with the candidate agent, wherein a change in the level of contactin expression indicates that the agent is suitable for use in treating patients having a neurodegenerative disease.

It is another aspect of the present invention to provide a cybrid cell line comprising immortal and differentiatable cells having genomic and mitochondrial DNA of differing biological origins, wherein the cells express contactin. In certain embodiments the cells are neural cells, and in certain other embodiments the cells are human cells. In certain other embodiments the cells are human central nervous system cells.

In still another aspect, the invention provides a method of identifying an agent capable of altering contactin expression in a cell comprising comparing the level of contactin expression in at least one cell before and after contacting the at least one cell with a candidate agent, and therefrom identifying an agent capable of altering contactin expression. In certain embodiments, the candidate agent comprises a test compound that is a small molecule, a nucleic acid molecule, an antisense nucleic acid molecule or a ribozyme. In another embodiment, the at least one cell comprises a cybrid cell. In other embodiments, the invention provides an agent capable of altering

contactin, as identified by the method just described. In certain embodiments, the agent is a small molecule, a protein, a polypeptide, an antibody, a nucleic acid molecule, an antisense molecule or a ribozyme. In other embodiments, the invention provides a pharmaceutical composition comprising the agent in a pharmaceutically acceptable carrier. It is further an aspect of the invention to provide a method of treating a patient having a neurodegenerative disease, comprising administering to the patient an effective amount of such pharmaceutical composition.

The present invention also provides, in another aspect, a method of treating a patient having a neurodegenerative disease, comprising administering to the patient an effective amount of a pharmaceutical composition capable of altering contactin expression. In certain embodiments, the invention provides a pharmaceutical target comprising a cellular component that binds to the agent capable of altering contacting expression, as described above. In certain other embodiments, the invention provides a method of identifying a pharmaceutical target, comprising contacting a compound that modulates contactin expression or activity with a biological sample known to exhibit contactin expression or activity.

In other embodiments the invention provides method for determining the risk for or presence of Alzheimer's disease or another neurodegenerative disease in a first subject suspected of having or being at risk for having such a disease, comprising determining the presence or absence of at least one differentially expressed nucleic acid molecule that is associated with Alzheimer's disease or another neurodegenerative disease in each of a first and a second biological sample comprising mitochondrial DNA, said first biological sample being obtained from said first subject and said second sample being obtained from a second subject known to be free of a risk or presence of a disease associated with altered mitochondrial function, wherein the presence of at least one differentially expressed nucleic acid molecule that is associated with Alzheimer's disease or another neurodegenerative disease in said first biological sample and the absence of a corresponding differentially expressed nucleic acid molecule having a corresponding nucleotide sequence in said second biological sample indicates an

increased risk of Alzheimer's disease, and therefrom determining the risk or presence of Alzheimer's disease or another neurodegenerative disease. In certain further embodiments, the step of determining comprises contacting each of said first and second biological samples with a nucleic acid array comprising a plurality of isolated nucleic acid molecules immobilized on a solid support, wherein said isolated nucleic acid molecules comprise at least one differentially expressed nucleic acid molecule that is associated with Alzheimer's disease or another neurodegenerative disease, under conditions and for a time sufficient to allow hybridization of DNA from said samples to said isolated nucleic acid molecules; and comparing an amount of hybridization to the nucleic acid array of (i) the nucleic acid molecule that is differentially expressed and that is associated with Alzheimer's disease or another neurodegenerative disease of the first sample, to (ii) an amount of hybridization of the nucleic acid of the second sample that corresponds to the nucleic acid molecule that is differentially expressed and that is associated with Alzheimer's disease or another neurodegenerative disease in the first sample, and therefrom determining the presence or absence of at least one differentially expressed nucleic acid molecule that is associated with Alzheimer's disease or another neurodegenerative disease.

These and other aspects of the present invention will become evident upon reference to the following detailed description. In addition, various references are set forth herein which describe in more detail certain aspects of this invention, and are therefore incorporated by reference in their entirety.

DETAILED DESCRIPTION OF THE INVENTION

The present invention is directed in part to the unexpected finding that expression levels of certain novel molecules described herein and of certain known molecules correlate with one or more neurodegenerative diseases, as described in greater detail below. As noted above, although abundant reference is made herein to altered expression levels of nucleic acid molecules that encode contactin as such a representative example, it is to be understood that the present invention relates as well

to the several additional nucleic acid molecules described herein that encode other products, including products having novel sequences and products having known sequences. The present invention is thus directed in part to the surprising observation that the GPI-linked, neuronal cell associated recognition molecule contactin (as a representative example) is associated with neurodegenerative diseases. In certain embodiments neurodegenerative disease may be correlated with altered (*e.g.*, increased or decreased in a statistically significant manner) levels of contactin. For example, as provided herein altered levels of contactin expression may be observed as increases or decreases in the amount of contactin protein in a sample relative to a control sample. As another example, altered levels of contactin may be detected as altered amounts of contactin mRNA, as also described in greater detail below.

The invention thus is directed in part to a method of detecting the risk for having or presence of a neurodegenerative disease in a subject, by comparing the level of contactin expression, such as the amount of contactin protein or contactin mRNA, in a test sample to the amount of contactin protein or mRNA in a control sample from a second subject known to be free of a risk for having or presence of the neurodegenerative disease. The invention also relates to a method of correlating contactin expression with the suitability of an agent for treating a neurodegenerative disease in at least one subject, thereby providing a method for identifying those patients having a neurodegenerative disease who are likely to respond to a particular treatment.

The invention is also directed in part to an oligonucleotide primer capable of specifically amplifying DNA or RNA encoding contactin or a nucleic acid sequence complementary thereto. Such primers may be nucleic acid molecules that can function as PCR primers for the detection of contactin mRNA or contactin DNA in a sample. Such nucleic acid molecules can also be labeled, and may be provided in a kit.

In view of the surprising relationship between contactin and neurodegenerative disease, the present invention further provides compositions and methods for drug screening assays, including the use of cybrid cells, to identify agents that may be useful for the treatment of neurodegenerative diseases, for example when

formulated into pharmaceutical compositions as provided herein. Similarly, the present disclosure provides methods for identifying molecular targets for pharmaceutical agents that alter contactin expression levels. as well as related therapeutic methods.

Neurodegenerative diseases to which the present invention may be applied include, but are not limited to. Alzheimer's disease (AD), multiple sclerosis (MS), Parkinson's disease (PD), diffuse Lewy body disease (DLB), vascular dementia and the like, and other neurodegenerative diseases.

Unless defined otherwise. all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention belongs. Generally, the nomenclature used herein and the laboratory procedures in cell culture, chemistry, microbiology, molecular biology, cell science and cell culture described below are well known and commonly employed in the art. Conventional methods are used for these procedures, such as those provided in the art and various general references (Sambrook et al., *Molecular Cloning: A Laboratory Manual, 2nd edition*, Cold Spring Harbor Press, Cold Spring Harbor, N.Y. (1989)). Where a term is provided in the singular, the inventors also contemplate the plural of that term. The nomenclature used herein and the laboratory procedures described below are those well known and commonly employed in the art. As employed throughout the disclosure, the following terms, unless otherwise indicated, shall be understood to have the following meanings:

"Membrane permeant derivative" refers to a chemical derivative of a compound that increases membrane permeability of the compound. These derivatives are made better able to cross cell membranes because hydrophilic groups are masked to provide more hydrophobic derivatives. Also, the making groups can be designed to be cleaved from the compound within a cell to make the compound more hydrophilic once within the cell. Because the substrate is more hydrophilic than the membrane permeant derivative, it preferentially localizes within the cell (U.S. Patent No. 5,741,657 to Tsien et al., issued April 21, 1998).

"Isolated polynucleotide" refers to a polynucleotide of genomic, cDNA, or synthetic origin, or some combination thereof, which by virtue of its origin, the isolated polynucleotide (1) is not associated with the cell in which the isolated polynucleotide is found in nature, or (2) is operably linked to a polynucleotide that it is not linked to in nature. The isolated polynucleotide can optionally be linked to promoters, enhancers, or other regulatory sequences.

"Isolated protein" refers to a protein of cDNA, recombinant RNA, or synthetic origin, or some combination thereof, which by virtue of its origin the isolated protein (1) is not associated with proteins normally found within nature, or (2) is isolated from the cell in which it normally occurs, or (3) is isolated free of other proteins from the same cellular source, for example, free of cellular proteins), or (4) is expressed by a cell from a different species, or (5) does not occur in nature.

"Polypeptide" is used herein as a generic term to refer to native protein, fragments, or analogs of a polypeptide sequence.

"Active fragment" refers to a fragment of a parent molecule, such as an organic molecule, nucleic acid molecule, or protein or polypeptide, or combinations thereof, that retains at least one activity of the parent molecule.

"Naturally occurring" refers to the fact that an object can be found in nature. For example, a polypeptide or polynucleotide sequence that is present in an organism, including viruses, that can be isolated from a source in nature and which has not been intentionally modified by man in the laboratory is naturally occurring.

"Operably linked" refers to a juxtaposition wherein the components so described are in a relationship permitting them to function in their intended manner. A control sequence operably linked to a coding sequence is ligated in such a way that expression of the coding sequence is achieved under conditions compatible with the control sequences.

"Control sequences" refer to polynucleotide sequences that effect the expression of coding and non-coding sequences to which they are ligated. The nature of such control sequences differs depending upon the host organism; in prokaryotes, such

control sequences generally include promoter, ribosomal biding site, and transcription termination sequences; in eukaryotes, generally, such control sequences include promoters and transcription termination sequences. The term control sequences is intended to include components whose presence can influence expression, and can also include additional components whose presence is advantageous, for example, leader sequences and fusion partner sequences.

"Polynucleotide" refers to a polymeric form of nucleotides of a least ten bases in length, either ribonucleotides or deoxynucleotides or a modified from of either type of nucleotide. The term includes single and double stranded forms of DNA or RNA.

"Genomic polynucleotide" refers to a portion of the genome.

"Active genomic polynucleotide" or active portion of a gnome" refer to regions of a gnome that can be up regulated, down regulated or both, either directly or indirectly, by a biological process.

"Directly" in the context of a biological process or processes, refers to direct causation of a process that does not require intermediate steps, usually caused by one molecule contacting or binding to another molecule (the same type or different type of molecule). For example, molecule A contacts molecule B, which causes molecule B to exert effect X that is part of a biological process.

"Indirectly" in the context of a biological process or precesses, refers to indirect causation that requires intermediate steps, usually caused by two or more direct steps. For example, molecule A contacts molecule B to exert effect X which in turn causes effect Y.

"Sequence homology" refers to the proportion of base matches between two nucleic acid sequences or the proportion of amino acid matches between two amino acid sequences. When sequence homology is expressed as a percentage, for example 50%, the percentage denotes the proportion of matches of the length of sequences from a desired sequence that is compared to some other sequence. Gaps (in either of the two sequences) are permitted to maximize matching; gap lengths of 15 bases or less are

usually used, 6 bases or less are preferred with 2 bases or less more preferred. When using oligonuleotides as probes or treatments, the sequence homology between the target nucleic acid and the oligonucleotide sequence is generally not less than 17 target base matches out of 20 possible oligonucleotide base pair matches (85%); preferably not less than 9 matches out of 10 possible base pair matches (90%), and most preferably not less than 19 matches out of 20 possible base pair matches (95%).

"Selectively hybridize" refers to detectably and specifically bind. Polynucleotides, oligonucleotides and fragments thereof selectively hybridize to target nucleic acid strands, under hybridization and wash conditions that minimize appreciable amounts of detectable binding to nonspecific nucleic acids. High stringency conditions can be used to achieve selective hybridization conditions as known in the art. Generally, the nucleic acid sequence homology between the polynucleotides, oligonucleotides, and fragments thereof and a nucleic acid sequence of interest will be at least 30%, and more typically and preferably of at least 40%, 50%, 60%, 70%, 80% or 90%.

Hybridization and washing conditions are typically performed at high stringency according to conventional hybridization procedures. Positive clones are isolated and sequenced. For example, a full length polynucleotide sequence can be labeled and used as a hybridization probe to isolate genomic clones from an appropriate target library as they are known in the art. Typical hybridization conditions and methods for screening plaque lifts and other purposes are known in the art (Benton and Davis, Science 196:180 (1978); Sambrook et al., supra, (1989)).

Two amino acid sequences are homologous if there is a partial or complete identity between their sequences. For example, 85% homology means that 85% of the amino acids are identical when the two sequences are aligned for maximum matching. Gaps (in either of the two sequences being matched) are allowed in maximizing matching; gap lengths of 5 or less are preferred with 2 or less being more preferred. Alternatively and preferably, two protein sequences (or polypeptide sequences derived from them of at least 30 amino acids in length) are homologous, as

this term is used herein, if they have an alignment score of at least 5 (in standard deviation units) using the program ALIGN with the mutation data matrix and a gap penalty of 6 or greater (Dayhoff. in Atlas of Protein Sequence and Structure, National Biomedical Research Foundation. volume 5, pp. 101-110 (1972) and Supplement 2, pp. 1-10). The two sequences or parts thereof are more preferably homologous if their amino acids are greater than or equal to 30% identical when optimally aligned using the ALIGN program.

"Corresponds to" refers to a polynucleotide sequence is homologous (for example is identical, not strictly evolutionarily related) to all or a portion of a reference polynucleotide sequence, or that a polypeptide sequence is identical to all or a portion of a reference polypeptide sequence. In contradistinction, the term "complementary to" is u sed herein to mean that the complementary sequence is homologous to all or a portion of a reference polynucleotide sequence. For illustration, the nucleotide sequence TATAC corresponds to a reference sequence TATAC and is complementary to a reference sequence GTATA.

The following terms are used to describe the sequence relationships between two or more polynucleotides: "reference sequence," "comparison window," "sequence identity," "percentage of sequence identity," and "substantial identity." A reference sequence is a defined sequence used as a basis for a sequence comparison; a reference sequence can be a subset of a larger sequence, for example, as a segment of a full length cDNA or gene sequence given in a sequence listing, or may comprise a complete cDNA or gene sequence. Generally, a reference sequence is at least 20 nucleotides in length, frequently at least 25 nucleotides in length, and often at least 50 nucleotides in length. Since two polynucleotides can each (1) comprise a sequence (for example a portion of the complete polynucleotide sequence) that is similar between the two polynucleotides, and (2) may further comprise a sequence that is divergent between the two polynucleotides, sequence comparisons between two (or more) polynucleotides are typically performed by comparing sequences of the two polynucleotides over a "comparison window" to identify and compare local regions of sequence similarity. A

comparison window, as used herein, refers to a conceptual segment of at least 20 contiguous nucleotide positions wherein a polynucleotide sequence may be compared to a reference sequence of at least 20 contiguous nucleotides and wherein the portion of the polynucleotide sequence in the comparison window can comprise additions and deletions (for example, gaps) of 20 percent or less as compared to the reference sequence (which would not comprise additions or deletions) for optimal alignment of the two sequences. Optimal alignment of sequences for aligning a comparison window can be conducted by the local homology algorithm (Smith and Waterman, *Adv. Appl. Math.*, 2:482 (1981)), by the homology alignment algorithm (Needleman and Wunsch, *J. Mol. Biol.*, 48:443 (1970)), by the search for similarity method (Pearson and Lipman, *Proc. Natl. Acid. Sci. U.S.A.* 85:2444 (1988)), by the computerized implementations of these algorithms such as GAP, BESTFIT, FASTA and TFASTA (Wisconsin Genetics Software Page Release 7.0, Genetics Computer Group, Madison, WI), or by inspection. Preferably, the best alignment (for example, the result having the highest percentage of homology over the comparison window) generated by the various methods is selected.

"Sequence identity" means that two polynucleotide sequences are identical (for example, on a nucleotide-by-nucleotide basis) over the window of comparison.

"Percentage of sequence identity" is calculated by comparing two optimally aligned sequences over the window of comparison, determining the number of positions at which the identical nucleic acid base occurs in both sequences to yield the number of matched positions, dividing the number of matched positions by the total number of positions in the window of comparison (for example, the window size), and multiplying the result by 100 to yield the percentage of sequence identity.

"Substantial identity" as used herein denotes a characteristic of a polynucleotide sequence, wherein the polynucleotide comprises a sequence that has at least 30 percent sequence identity, preferably at least 50 to 60 percent sequence identity, more usually at least 60 percent sequence identity as compared to a reference sequence over a comparison window of at least 20 nucleotide positions, frequently over a window

of at least 25 to 50 nucleotides, wherein the percentage of sequence identity is calculated by comparing the reference sequence to the polynucleotide sequence that may include deletions or additions which total 20 percent or less of the reference sequence over the window of comparison.

"Substantial identity" as applied to polypeptides herein means that two peptide sequences, when optimally aligned, such as by the programs GAP or BESTFIT using default gap weights, share at least 30 percent sequence identity, preferably at least 40 percent sequence identity, and more preferably at least 50 percent sequence identity, and most preferably at lest 60 percent sequence identity. Preferably, residue positions, which are not identical, differ by conservative amino acid substitutions.

"Conservative amino acid substitutions" refer to the interchangeability of residues having similar side chains. For example, a group of amino acids having aliphatic side chains is glycine, alanine, valine, leucine, and isoleucine; a group of amino acids having aliphatic-hydroxyl side chains is serine and threonine; a group of amino acids having amide-containing side chains is asparagine and glutamine; a group of amino acids having aromatic side chains is phenylalanine, tyrosine and tryptophan; a group of amino acids having basic side chains is lysine, arginine and histidine; and a group of amino acids having sulfur-containing side chan is cysteine and methionine. Preferred conservative amino acid substitution groups are: valine-leucine-isoleucine; phenylalanine-tyrosine; lysine-arginine; alanine-valine; glutamic-aspartic; and asparagine-glutamine.

"Modulation" refers to the capacity to either enhance or inhibit a functional property of a biological activity or process, for example, enzyme activity or receptor binding. Such enhancement or inhibition may be contingent on the occurrence of a specific event, such as activation of a signal transduction pathway and/or may be manifest only in particular cell types.

"Modulator" refers to a chemical (naturally occurring or non-naturally occurring), such as a biological macromolecule (for example, nucleic acid, protein, non-peptide or organic molecule) or an extract made from biological materials, such as

prokaryotes, bacteria, eukaryotes, plants, fungi, multicellular organisms or animals, invertebrates, vertebrates, mammals and humans, including, where appropriate, extracts of: whole organisms or portions of organisms, cells, organs, tissues, fluids, whole cultures or portions of cultures, or environmental samples or portions thereof. Modulators are typically evaluated for potential activity as inhibitors or activators (directly or indirectly) of a biological process or processes (for example, agonist, partial antagonist, partial agonist, antagonist, antineoplastic, cytotoxic, inhibitors of neoplastic transformation or cell proliferation, cell proliferation promoting agents, antiviral agents, antimicrobial agents, antibacterial agents, antibiotics, and the like) by inclusion in assays described herein. The activity of a modulator may be known, unknown or partially known.

"Test compound" refers to a chemical, compound, composition or extract to be tested by at least one method of the present invention to be a putative modulator. Test compounds can be candidate agents and may include small molecules, such as small molecules, drugs, proteins or peptides or active fragments thereof, such as antibodies, nucleic acid molecules such as DNA, RNA or combinations thereof, antisense molecules or ribozymes, or other organic or inorganic molecules, such as lipids, carboydrates, or any combinations thereof. Test compounds that include nucleic acid molecules can be provided in a vector, such as a viral vector, such as a retrovirus, adenovirus or adeno-associated virus, a liposome, a plasmid or with a lipofection agent. Test compounds, once identified, can be agonists, antagonists, partial agonists or inverse agonists of a target. A test compound is usually not known to bind to the target of interest. "Control test compound" refers to a compound known to bind to the target (for example, a known agonist, antagonist, partial agonist or inverse agonist). Test compound does not typically include a compound added to a mixture as a control condition that alters the function of the target to determine signal specificity in an assay. Such control compounds or conditions include chemicals that (1) non-specifically or substantially disrupt protein structure (for example denaturing agents such as urea or guandium, sulfhydryl reagents such as dithiotritol and beta-mercaptoethanol), (2)

generally inhibit cell metabolism (for example mitochondrial uncouples) and (3) non-specifically disrupt electrostatic or hydrophobic interactions of a protein (for example, high salt concentrations or detergents at concentrations sufficient to non-specifically disrupt hydrophobic or electrostatic interactions). The term test compound also does not typically include compounds known to be unsuitable for a therapeutic use for a particular indication due to toxicity of the subject. Usually, various predetermined concentrations of test compounds are used for determining their activity. If the molecular weight of a test chemical is known, the following ranges of concentrations can be used: between about 0.001 micromolar and about 10 millimolar, preferably between about 0.01 micromolar and about 1 millimolar, more preferably between about 0.1 micromolar and about 100 micromolar. When extracts are uses a test compounds, the concentration of test chemical used can be expressed on a weight to volume basis. Under these circumstances, the following ranges of concentrations can be used: between about 0.001 micrograms/ml and about 1 milligram/ml, preferably between about 0.01 micrograms/ml and about 100 micrograms/ml, and more preferably between about 0.1 micrograms/ml and about 10 micrograms/ml.

"Target" refers to a biochemical entity involved in a biological process. Targets are typically proteins that play a useful role in the physiology or biology of an organism. A therapeutic composition or compound typically binds to a target to alter or modulate its function. As used herein, targets can include, but not be limited to, cell surface receptors, G-proteins, G-protein coupled receptors, kinases, phosphatases, ion channels, lipases, phosholipases, nuclear receptors, intracellular structures, tubules, tubulin, and the like.

"Label" or "labeled" refers to incorporation of a detectable marker, for example by incorporation of a radiolabled compound or attachment to a polypeptide of moieties such as biotin that can be detected by the binding of a section moiety, such as marked avidin. Various methods of labeling polypeptide, nucleic acids, carbohydrates, and other biological or organic molecules are known in the art. Such labels can have a variety of readouts, such as radioactivity, fluorescence, color, chemiluminescence or

other readouts known in the art or later developed. The readouts can be based on enzymatic activity, such as beta-galactosidase, beta-lactamase, horseradish peroxidase, alkaline phosphatase, luciferase; radioisotopes such as ^{3}H, ^{14}C, ^{35}S, ^{125}I or ^{131}I; fluorescent proteins, such as green fluorescent proteins; or other fluorescent labels, such as FITC, rhodamine, and lanthanides. Where appropriate, these labels can be the product of the expression of reporter genes, as that term is understood in the art. Examples of reporter genes are beta-lactamase (U.S. Patent No. 5,741,657 to Tsien et al., issued April 21, 1998) and green fluorescent protein (U.S. Patent No. 5,777,079 to Tsien et al., issued July 7, 1998; U.S. Patent No. 5,804,387 to Cormack et al., issued September 8, 1998).

"Substantially pure" refers to an object species or activity that is the predominant species or activity present (for example on a molar basis it is more abundant than any other individual species or activities in the composition) and preferably a substantially purified fraction is a composition wherein the object species or activity comprises at least about 50 percent (on a molar, weight or activity basis) of all macromolecules or activities present. Generally , as substantially pure composition will comprise more than about 80 percent of all macromolecular species or activities present in a composition, more preferably more than about 85%, 90%, 95% and 99%. Most preferably, the object species or activity is purified to essential homogeneity, wherein contaminant species or activities cannot be detected by conventional detection methods) wherein the composition consists essentially of a single macromolecular species or activity. The inventors recognize that an activity may be caused, directly or indirectly, by a single species or a plurality of species within a composition, particularly with extracts.

"Pharmaceutical agent or drug" refers to a chemical, composition or activity capable of inducing a desired therapeutic effect when property administered by an appropriate dose, regime, route of administration, time and delivery modality.

"Pharmaceutically effective amount" refers to an appropriate dose, regime, route of administration, time and delivery modality associated with the delivery

of an amount of a compound or composition to cause a desired effect. Such pharmaceutically effective amount can be determined using methods described herein or by the United States Food and Drug Administration (USFDA).

"Sample" means any biological sample, preferably derived from a test animal, such as a mouse, rat, rabbit or monkey, or a patient, such as a human. Samples can be from any tissue or fluid, such as neural tissues, central nervous tissues, internal organs such as pancreas, liver, lung, kidney, muscle, skeletal muscle, urine, feces, blood, fluids from body cavities or the central nervous system, or samples from various body cavities such as the mouth or nose. Samples derived from urine and feces contain cells of the immunological, urinary or digestive tract and can be a rich source of sample. Such samples can be obtained using methods known in the art, such as biopsies, aspirations, scrapings or simple collection. A sample can be taken from a test animal or patient that is either living or dead.

"Ribozyme" means enzymatic RNA molecules capable of catalyzing the specific cleavage of RNA. The mechanism of ribozyme action involves sequence-specific hybridization of the ribozyme molecule to complementary target RNA, followed by endonucleolytic cleavage. Within the scope of the invention are engineered hammerhead motif ribozyme molecules that can specifically and efficiently catalyze endonucleolytic cleavage of RNA encoding contactin. Specific ribozyme cleavage sites within any potential RNA target are initially identified by scanning the target RNA target for ribozyme cleavage sites which include the sequences GUA, GUU and GUC. Once identified, short RNA sequences between 15 and 20 ribonucleotides corresponding to the region of the target gene containing the cleavage site can be evaluated for secondary structural features which may render the oligonucleotide inoperable. The suitability of candidate targets can also be evaluate by testing accessibility to hybridization with complementary oligonucleotides using ribonuclease protection assays.

"Contactin" refers to contactin mRNA, contactin DNA and contactin protein. "Contactin protein" means a protein that exhibits at least one activity of at least

one human contactin, F3 or F11. "Contactin mRNA" is a mRNA molecule that encodes a contactin protein. Preferably, the contactin mRNA is derived from nuclear DNA, but it can be derived from mitochondrial DNA "Contactin DNA" is a DNA molecule that encodes a contactin protein. Preferably a contactin DNA is a nuclear DNA, but it can be a mitochondrial DNA.

Other technical terms used herein have their ordinary meaning in the art that they are used, as exemplified by a variety of technical dictionaries, such as the McGraw-Hill Dictionary of Chemical Terms and the Stedman's Medical Dictionary.

As noted above, the present invention recognizes that contactin expression (as a representative example. expression of other novel and known products described herein being within the scope of the instant invention as noted above) in cells is related to neurodegenerative diseases. As a non-limiting introduction to the breadth of the present invention, the present invention includes several general and useful aspects, including (i) nucleic acid molecules that can function as PCR primers for the detection of contactin mRNA or contactin DNA in a sample; (ii) methods for detecting the presence of a neurodegenerative disease such as multiple sclerosis, AD, PD, DLB or other neurodegenerative disease as provided herein, including providing a sample from a patient and measuring the amount of contactin protein, contactin DNA or contactin mRNA expressed in the sample; (iii) methods for identifying which patients having a neurodegenerative disease are likely to respond to a treatment for a neurodegenerative disease; (iv) cells and cell lines, including cybrid cell lines, that express a normal, increased or decreased amount of contactin as compared to a control cell; (v) methods for screening compounds for the ability to alter (*e.g.*, increase or decrease) the expression of contactin and compositions or compounds, including pharmaceutical compositions, identified by these methods, that reduce or enhance contactin expression; (vi) methods of treating a neurodegenerative disease using the compositions or compounds identified by the methods of the present invention; and (vii) methods for identifying pharmaceutical targets for compounds that alter or modulate the amount of

contactin DNA, contactin mRNA or contactin protein in a cell and targets identified by methods of the present invention.

These aspects of the invention, as well as others described herein, can be achieved by using the methods, articles of manufacture and compositions of matter described herein. To gain a full appreciation of the scope of the present invention, it will be further recognized that various aspects of the present invention can be combined to make desirable embodiments of the invention.

Nucleic Acid Molecules

The present invention includes nucleic acid molecules that can function as PCR primers for the detection of contactin mRNA or contactin DNA in a sample (or of other nucleic molecules encoding other products as described herein). Such nucleic acid molecules can be detectably labeled and can be provided in a kit.

Human contactin protein, and its homologs from mouse (F3 protein) and chicken (F11 protein), is a cell surface protein that is related to cell attachment to substrate. Contactin comprises a Ig-like domain and multiple fibronectin III-like domains. Rather than being a transmembrane protein, contactin attaches to glycosylphosphatidylinositol (GPI) on the outer membrane of the cell surface. Relatively high levels of a contactin mRNA (6.5 kb) are expressed in adult brain with three minor transcripts (9.7 kb, 4.4 kb and 3.4 kb), whereas low levels of expression of multiple forms of contactin mRNA are found in the adult lung, pancreas, kidney and skeletal muscles (6.8 kb and 6.0 kb). High levels of expression of the multiple forms of contactin mRNA are found in the cancerous cells neuroblastoma and retinoblastoma (6.8 kb, 6.0 kb and 4.2 kb). The expression of contactin in developing neural tissue is complex and transient, and is believed to have a role in neurite outgrowth, binding to the cell recognition molecule Ng-CAM and interaction with the extracellular matrix glycoprotein restrictin. Adult neural stem cells can give rise to hematopoietic cells, myeloid cells and lymphoid cells (Bjorson et al., Science 283:534 etc. (1998)). Thus, contactin mRNA, contactin DNA or contactin protein may be detected in blood.

A variety of human contactin mRNA sequences have been reported. Reid and Hemperly report a human contactin mRNA having 3360 nucleotides, accession number Z21488 (Reid and Hemperly, Bran Res. 21:1-8 (1994)). Berglund and Ranscht report an mRNA encoding human contactin 1 precursor having 3314 nucleotides, accession number U07819 (Berglund and Ranscht, Genomics 21:571-582 (1994)). Berglund and Ranscht report an mRNA encoding human contactin 2 precursor having 3335 nucleotides, accession number U07820 (Berglund and Ranscht, Genomics 21:571-582 (1994)). Watanabe et al. report a mRNA encoding bovine F3/F11/contactin having 3412 nucleotides (Watanabe et al., Gene 160:245-248 (1995)). Hosoya et al. report a mRNA encoding rat F3 having 3214 nucleotides (Hosoya et al., Neurosci, Lett. 186:2-3 (1995)). Portions of the sequences of Neuro-1, human contactin, mouse F3 and chicken F11 have been compared (Reid et al., Mol. Brain Res. 21:1-8 (1994)). Also, PCR primers for mouse F3 have been reported (Reid et al., Mol. Brain Res. 21:1-8 (1994)). The functional domains of chicken F11 have been mapped using deletions of various regions of chicken F11 (Brummendorf et al., Neuron 10:711-727 (1993)). These functional domains have been compared to surface molecules having similar functions (Brummendorf and Rathjen , J. Neurochem. 61:1207-1219 (1993)).

The inventors contemplate that there are allelic variants of the various contactins which may have sequences different from those reported in the literature. Allelic variants may have different sequences from the reported sequences, such as those that arise naturally from deletions, insertions or substitutions. Allelic variants may have different structures or different functions from the contactins reported in the literature. Such allelic variants are considered by the inventors to encode contactin.

In addition to allelic variants, the inventors contemplate altered nucleic acid sequenced that encode a contactin including deletions, insertions or substitutions of different nucleotides resulting in a polynucleotide that encodes the same, a functionally equivalent contactin or a contactin that retains at least one activity of the parent contactin. Such altered nucleic acid sequences can be made using established methods in the art, such as site directed mutagenesis or random mutagenesis as they are known in

the art. Proteins encoded by such altered nucleic acid sequences may exhibit equivalent, similar or different structures or activities as compared to the parent molecule. Such altered nucleic acid sequences are considered by the inventors to encode contactin.

The present invention includes nucleic acid molecules that are useful as primers for use in PCR amplification procedures specific for the amplification of at least one contactin mRNA or contactin DNA, particularly in samples derived from humans (see, U.S. Patent No. 4,683,195; U.S. Patent No. 4,965,188; and Innis et al., *PCR Strategies*, Academic Press, San Diego (1995) for PCR procedures). Such PCR amplification methods are known in the art and include primer extension PCR, real time PCR, reverse transcriptase PCR, (Freeman et al., *BioTechniques* 26:112-125 (1999)) inverse PCR (Triglia et al., *Nucleic Acids Res.* 16:8186 (1988)), capture PCR (Lagerstrom et al., *PCR Methods Applic.* 1:111-119 (1991)), differential primer extensions (WO 96/30545 to Fahy and Ghosh, published October 3, 1996) and other PCR amplification methods known in the art or later developed (see, Innis et al., *PCR Strategies*, Academic Press, San Diego (1995)).

In operation, PCR methods generally use primer molecules that are usually chemically synthesized, but they may be generated enzymatically or produced from a recombinant course. PCR primers generally comprise two nucleotide sequences, one with sense orientation (5' -> 3') and one with antisense (3' -> 5'), employed under preferred conditions for identification of a specific gene or condition. The same PCR primers, nested sets of oligomers or a degenerate pool of oligomers can be employed under less stringent conditions for detection and/or quantitation of closely related DNA or RNA sequences.

Additionally, methods that can be used to quantitate the expression of a particular molecule include radiolabeling (Melby et al., J. Immunol Methods 159:235-244 (1993)) or biotinylating (Duplaa et al., Anal. Biochem. 229-236 (1993)) nucleotides, coamplification of a control nucleic acid, and standard curves onto which the experimental results are interpolated. Quantitation of multiple samples can be sped

up by running the assay in an ELISA format where the nucleic acid molecule of interest is presented in various dilutions and a spectrophotometric or colorimetric responses gives rapid quantitation. Colorimetric intercalating dyes can be used in such quantitative methods, as is described in the Examples and as are known in the art (Freeman et al., *BioTechniques*, 26:112-125 (1999); and Spiess et al., *BioTechniques* 26:46-50 (1999)).

These nucleic acid molecules can also be used individually or in combination as probes to identify contactin mRNA or DNA molecules in a sample. These nucleic acid molecules include

5'-TCAGTAAGGTCTGGTTCACGCTAT-3' (SEQ ID NO:1),

5'-TCCCGTCACTGTAGATTCATTTGA-3' (SEQ ID NO:2),

5'-CCCCAAGTCTTCTCGGCTTA-3' (SEQ ID NO:3),

5'-CAACACATTCAGAATTCCAAGTAGACA-3' (SEQ ID NO:4),

5'-TCCCCAAGTCTTCTCGGCTTA-3' (SEQ ID NO:5),

5'-CCCATCCCAGCTCAGAAGAC-3' (SEQ ID NO:6), and

5'-GCCGCAGAAATTGGAAGG-3' (SEQ ID NO:7).

SEQ ID NO: 1, SEQ ID NO:3, SEQ ID NO:5 and SEQ ID NO:6 are forward primers. SEQ ID NO:2, SEQ ID NO:4 and SEQ ID NO:7 are reverse primers.

The invention includes primers that have substantial identity to the nucleic acid molecules of SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, or SEQ ID NO:7 or portions thereof or antisense versions thereof, so long as they are capable of specifically amplifying contactin RNA or DNA in a sample.

Other primers can be utilized in such PCR procedures, so long as they are specific for the amplification of at least one human contactin mRNA or contactin DNA in a sample, particularly in samples derived from humans. Such PCR primers can be selected by identifying stretches of at least one contactin DNA or RNA that are unique to at least one contactin DNA or mRNA using sequence comparing algorithms, such as Altschul et al., *Nucleic Acids Res*. 25:3389-3402 (1997). The specificity of such primers to amplify at least one contactin mRNA or contactin DNA can be determined using the methods of the present invention as exemplified by the Examples. The invention includes primers that have substantial identity to such identified nucleic acid molecules or portions thereof, so long as they are capable of specifically amplifying at least one contactin mRNA or contactin DNA in a sample.

The present invention also includes antisense versions of these nucleic acid molecules. Such antisense molecules are useful as probes to detect DNA or RNA encoding contactin in a sample. Such antisense sequences can be determined by deducing the antisense sequence from a given sequence. Such antisense molecules can be either DNA or RNA, and the skilled artisan would of course appreciate the different base pairs used in the coding scheme for these different nucleic acid molecules. These antisense molecules can be used to modulate gene expression of the targeted sequence. Preferably, such antisense molecules target the transcription initiation site of a gene to prevent transcription, or a mRNA molecule, to present translation or binding of ribosomes to the mRNA. Inhibition of the transcription or translation of a gene can also be accomplished using "triple helix" base-pairing methodologies, which comprises the ability of double helix to open sufficiently for the binding of polymerases, transcription factors, or regulatory molecules. Recent therapeutic advances using triplex DNA were reviewed by Gee et al. (In: Huber and Carr, *Molecular and Immunologic Approaches*, Futura Publishing Co., N.Y. (1994)).

The nucleic acid molecules of the present invention can be made by a variety of methods known in the art. For example, nucleic acid molecules can be made

using synthetic procedures or molecular biology techniques known in the art (see, Sambrook et al., supra).

The length of the nucleic acid molecules of the present invention can be readily chosen by one skilled in the art depending on the particular purpose that the nucleic acid molecule is to be used for. For PCR primers, the length of the nucleic acid molecule is preferably between about 10 nucleotides and about 50 nucleotides in length, more preferably between about 12 nucleotides and about 30 nucleotides in length, and most preferably between about 15 nucleotides and about 20 nucleotides in length. For probes, the length of the nucleic acid molecule is preferably between about 20 nucleotides and about 1,000 nucleotides in length, more preferably between about 100 nucleotides in length and about 500 nucleotides in length, and most preferably between about 200 nucleotides and about 400 nucleotides in length.

The nucleic acid molecules of the present invention can be linked to a detectable label to form a labeled nucleic acid molecule.. Such labeled nucleic acid molecules can be made using methods known in the art. Such labeled nucleic acid molecules are useful alone or in combination as probes to detect contactin DNA or contactin mRNA in a sample using established nucleic acid hybridization methods, such as solid-phase hybridizations or *in situ* hybridizations. Such labeled probes can be used in PCR procedures that utilize labeled primers, included multiple labeled primers, such as fluorescence resonance energy transfer (FRET) based amplification procedures as they are known in the art.

The labeled or unlabeled nucleic acids of the present invention can be provided separately or in combination in a kit to practice at least one method of the present invention. The nucleic acid molecules can be provided in a single or separate container, along with other reagents, buffers or materials to be used in practicing at least one method of the present invention. The kit can be provided in a container, such as a packaging container, that can optionally include instructions for performing at least one method of the present invention. The instructions can be provided in any language or

format, preferably in a language and format directed towards a target end-user such that the end user can practice at least one method of the present invention.

Methods for Detecting the Presence of a Neurodegenerative Disease

The present invention also includes methods for detecting the presence of a neurodegenerative disease such as multiple sclerosis, including providing a sample from a patient and measuring the amount of contactin protein, contactin DNA or contactin mRNA expressed in the sample.

Samples for use in this method can be taken from any tissue, organ or fluid from the patient. Such samples can be obtained using methods known in the art, such as biopsies, aspirations or scrapings. Preferred samples include samples from the nervous system, including the central nervous system, pancreas, lung, kidney, blood, mouth, nasal passages, urine, feces and skeletal muscle. Preferably, the sample is derived at least in part from the central nervous system of the patient. The samples can used as they are taken from the patient, or be processed by, for example, thin section preparation, homogenization, or for the culturing of cells within the sample using established methods. Once samples are prepared for a particular detection method, the amount of contactin mRNA, contactin DNA or contactin protein in a sample can be determined.

Contactin mRNA in a sample can be measured using a variety of established methods, such as PCR methods as they are known in the art. Such PCR methods utilize appropriate nucleic acid molecules of the present invention as they are discussed in the previous section and in the Examples. Contactin mRNA can also be measured using hybridization methods, such as blot analysis, such as Northern Blot analysis or slot/dot blot analysis, and *in situ* hybridization as such methods are known in the art

Contactin DNA can be measured using established methods, such as hybridization methods known in the art. Such hybridization methods would utilize appropriate nucleic acid molecules of the present invention as they are discussed in the previous section. Contactin DNA can also be measured using hybridization methods,

such as blot analysis, such as Southern Blot analysis or slot/dot blot analysis, or *in situ* hybridization as such methods are known in the art. Contactin DNA can also be determined in chromosomal or cellular preparation using fluorescence in situ hybridization (FISH) methods as they are known in the art using the nucleic acid molecules of the present invention (Verma et al., Human Chromosomes: A Manual of Basic Techniques, Pergamon Press, N.Y. (1988)).

Contactin protein in a sample can be determined using a variety of methods. For example, immunological methods, such as ELISA, Western Blot analysis, or immunocytochemical analysis can be utilized. Generally, these methods use a primary antibody that is specific for contactin. Such antibodies are known in the art, or can be made using established methods known in the art (Faivre-Sarrailh et al., J. Nurosci., 12:257-267 (1992); Brummendorf et al., Neuron 10:711-727 (1993); and Pesheva et al., Neuron 10:69-82 (1993)). This primary antibody can be attached to a detectable label so that the binding of the primary antibody to contactin in a sample can be detected. Alternatively, the primary antibody is not attached to a detectable label. In this instance, a secondary antibody that specifically binds with the primary antibody, preferably at the Fc region of the primary antibody, is used. The secondary antibody is attached to a detectable label so that the binding of the primary antibody to contactin is detected.

The amount of contactin mRNA, contactin DNA or contactin protein in a sample is then preferably compared to the amount of contactin mRNA, contactin DNA or contactin protein in a control sample. Appropriate control samples are readily chosen by the skilled artisan. For example, appropriate control samples include samples taken from normal patents that do not have a particular disease state or who are known to express a normal amount of contactin. Such a control would be derived from the same tissue, organ or fluid that the test sample was derived from so that a valid comparison can be made. The amount of contactin mRNA, contactin DNA or contactin protein in a control sample can be provided in the form of a chart or other documentation or database such that control samples need not be processed for all

assays. Differences between the amount of contactin mRNA, DNA or protein in the sample that are statistically different from the amount of contactin mRNA, DNA or protein in the control sample is indicative of a neurodegenerative disease, particularly from the group consisting of amyotrophic lateral sclerosis, multiple sclerosis, MELAS and MERRF.

The method of the present invention can also comprise measuring the amount of control mRNA, control DNA or control protein in the sample, wherein the control mRNA or control DNA does not encode contactin and the control protein is not contactin. Preferably, the control mRNA. control DNA or control protein relates to a protein that is constitutively, constantly or highly expressed, such as actin, ribosomal RNA or GAPDH. In this instance, the control is an internal control such that the amount of contactin mRNA, contactin DNA or contactin protein and the amount of control mRNA, control DNA or control protein are detected in the same sample. Control mRNA, control DNA or control protein can be detected using the methods described herein for detection of contactin mRNA, contactin DNA or contactin protein. The amount of control mRNA, control DNA or control protein are preferably compared to the amount of contactin mRNA, contactin DNA or contactin protein in the sample. Preferably, the ratio of contactin / control mRNA, DNA or protein is determined and compared to the normal values obtained from samples derived from normal samples. Differences in the ratio of control mRNA, DNA or protein that are statistically different from normal values is indicative of a neurodegenerative disease, particularly from the group consisting of amyotrophic lateral sclerosis, multiple sclerosis, MELAS and MERRF.

In certain other preferred embodiments, nucleic acid molecules having specific nucleotide sequences may be efficiently detected, screened and/or quantified by high throughput hybridization methodologies directed to independently probing a plurality of distinct DNAs, or a plurality of distinct oligonucleotide primers as provided herein, that have been immobilized as nucleic acid arrays on a solid phase support. Typically, the solid support may be silica, quartz or glass, or any other material on

which nucleic acid may be immobilized in a manner that permits appropriate hybridization, washing and detection steps as known in the art and as provided herein. In preferred embodiments, solid-phase nucleic acid arrays are precisely spatially addressed, as described, for example, U.S. Patent 5,800,992 (see also, *e.g.*, WO 95/21944; Schena et al., 1995 *Science* 270:467-470, 1995; Pease et al., *1994 Proc. Nat. Acad. Sci. USA* 91:5022; Lipshutz et al., 1995 *Biotechniques 19*: 442-447).

Detection of hybridized (*e.g.*, duplexed) nucleic acids on the nucleic acid array may be achieved according to any known procedure, for example, by spectrometry or potentiometry (*e.g.*, MALDI-MS). Within certain preferred embodiments the array contains oligonucleotides that are less than 5500 nt, in other preferred embodiments less than 500 nt, in other preferred embodiments less than 100 nt, and in other preferred embodiments less than 50 nt in length. For high throughput screening of nucleic acid arrays, the format is preferably amenable to automation. It is preferred, for example, that an automated apparatus for use according to high throughput screening embodiments of the present invention is under the control of a computer or other programmable controller. The controller can continuously monitor the results of each step of the nucleic acid deposition, washing, hybridization, detection and related processes, and can automatically alter the testing paradigm in response to those results.

Methods for Identifying Which Patients Having a Neurodegenerative Disease are Likely to Respond to a Treatment for a Neurodegenerative Disease

The present invention includes methods for identifying which patients having a neurodegenerative disease are likely to respond to a treatment for a neurodegenerative disease.Such methods include: providing samples from a group of patients having said neurodegenerative disease; measuring the amount of contactin protein, contactin mRNA or contactin DNA present in the samples; providing said treatment to said patients; measuring the degree, frequency, rate or extent of responses of said patients to said treatment; and determining if a correlation exists between the amount of contactin protein, contactin DNA or contactin mRNA present in said samples and the degree, frequency, rate or extent of said responses. Wherein: 1) if said

correlation is a positive correlation. the presence of said positive correlation indicates that the patients providing samples having an increased amount of contactin protein, contactin DNA or contactin mRNA are likely to respond to said treatment; or 2) if said correlation is a negative correlation, the presence of the negative correlation indicates that the patients providing samples having a decreased amount of contactin protein, contactin DNA or contactin mRNA are likely to respond to said treatment.

In the practice of this method, samples are provided from a group of patients that have been diagnosed as having a neurodegenerative disease, such as multiple sclerosis , amyotrophic lateral sclerosis, MELAS or MERRF. The sample can be from any tissue, organ or fluid, but is preferably derived at least in part from neurological tissue, preferably central nervous tissue. The group of patients is preferably greater than one, more preferably at least four, and most preferably at least nine. The amount of contactin mRNA, contactin DNA or contactin protein is measured in these samples using methods known in the art or described herein.

The patients are then provided a treatment that may regress, alleviate, reduce the severity, reduce the progress or cure the neurodegenerative disease. The treatment can be any treatment, including those that administer or do not administer a compound or composition. For example, the treatment can include the use of companion animals or humans, massage, humor or other treatment that does not include the purposeful administration of a compound or composition to the patent by any route of administration. The treatment can also include administering a compound or composition to the patient, including tradition or non-traditional medicines or treatments, such as herbal remedies or aroma therapy. The response of the patients are monitored using criteria and endpoints that are related to or established for the neurodegenerative disease. For example, the degree, frequency, rate or extent of the patients' response to the treatment can be measured using methods and endpoints established for the neurodegenerative disease. Should a patient die during the treatment, samples can be taken from the cadaver and death be recorded as the progress of the disease.

Samples are taken from the patents at least one time during the course of the treatment. Preferably, the samples are from the same tissue, organ or fluid that the original sample was taken from. The amount of contactin mRNA, contactin DNA or contactin protein is measured in these samples.

The response of the patients are correlated to the change in the amount of contactin mRNA, contactin DNA or contactin protein in the samples from the patients. A positive correlation exists if the presence of the correlation indicates that patients providing samples having an increased amount of contactin mRNA, contactin DNA or contactin protein are likely to respond to the treatment. A negative correlation exists if the presence of the correlation indicates that patients providing samples having a decreased amount of contactin mRNA, contactin DNA or contactin protein are likely to respond to the treatment.

For both positive and negative correlations, the response can be desirable or undesirable. A desirable response is a response that regresses, alleviates, reduces the severity, reduces the progress or cures the neurodegenerative disease. An undesirable response is any response that is not a desirable response.

Cells That Exhibit an Increased or Decreased Amount of Contactin

The present invention also includes compositions of matter that include a cell or a cell in culture that exhibit an increased or decreased amount of contactin, such as contactin mRNA, contactin DNA or contactin protein, as compared to a control cell. The cell can be part of a tissue, organ or fluid, or a portion thereof. The cell can also be cultured *in vitro*.

The cells of the present invention can be derived from a sample taken from a test animal or patient, such as a human patient. Such cells can be part of a primary cell culture or a continuous cell line. Preferably, the cells of the present invention are a clonal population. Primary cell cultures can be obtained using methods known in the art. Continuous cell lines can be made by repeated passage of a population of cells in culture until a continuous cell line is obtained. Alternatively, a primary cell can be made into a continuous cell line by immortalizing the cell line using

methods known in the art. For example. a primary cell can be fused with an immortal cell line using polyethylene glycol or an electric charge. Alternatively, a primary cell can be infected with a transforming virus, such as a retrovirus, to make the cell line immortal.

A cell can also be engineered using methods known in the art so that the cell exhibits an increased or decreased amount of contactin mRNA, contactin DNA or contactin protein. For example, cells that exhibit an increased amount of contactin mRNA, contactin DNA or contactin protein can be made by transfecting a cell line with nucleic acid molecules encoding a contactin protein. The nucleic acid molecule can be provided in a vector. and can be operably linked to control sequences, such as CMV promoters or LTR elements, such that relatively high levels of contactin are expressed in the cell. The nucleic acid molecule encoding a contactin can also be provided in a vector that is operably linked to a nucleic acid sequences that promote homologous recombination to known regions of the genome such that the nucleic acid molecule encoding a contactin can be expressed under the control of an endogenous promoter (see, WO 94/24301 to Smith et al., published October 27, 1994). The nucleic acid molecule encoding a contactin can also be provided in a vector that is not operably linked to a control sequences or nucleic acid sequences that promote homologous recombination such that the nucleic acid molecule encoding a contactin is randomly integrated into the genome of the cell (see, WO 98/13353 to Whitney, published April 2, 1998). The increased amount of contactin mRNA, contactin DNA or contactin protein in these cells can be confirmed using the methods of the present invention

Cells that exhibit a decreased amount of contactin can be made by transfecting cell lines with nucleic acid molecules that encode antisense molecules to contactin DNA or contactin mRNA or that encode ribozymes that can degrade contactin mRNA using methods known in the art. The decreased amount of contactin mRNA, contactin DNA or contactin protein in these cells can be confirmed using the methods of the present invention.

A cell that exhibits an increased or decreased amount of contactin mRNA, contactin DNA or contactin protein can be used to make a cybrid cell using methods known in the art (see, WO 95/26973 to Herrnstadt et al., published October 12, 1995). Briefly, a cell is made essentially devoid of mitochondria by exposure to ethidium bromide. These cells are then fused with platelets from a patient, such as a patent with a neurodegenerative disorder or a normal patient, which comprise mitochondria. The fused cell lines contain the nuclear elements of the cell and the mitochondria, including the mitochondrial DNA, of the platelets. The amount of contactin mRNA, contactin DNA or contactin in these cybrids can be measured using the methods of the present invention and can be compared to the cell prior to the fusion with platelets.

Furthermore, cybrids that exhibit an increased or decreased amount of contactin mRNA, contactin DNA or contactin protein can be made. A cell is exposed to ethidium bromide to make the cell essentially devoid of mitochondria. The cell essentially devoid of mitochondria (such as human SH-SY5Y neuroblastoma cell line) is fused with platelets from a normal patient or a patient with a neurodegenerative disorder. The amount of contactin mRNA, contactin DNA or contactin protein in these cybrids can be measured using the methods of the present invention and compared to the amount of contactin mRNA, contactin DNA or contactin protein expressed in the parent cells to identify cybrid cell lines that exhibit an increased or decreased amount of contactin mRNA, contactin DNA or contactin protein.

Methods for Screening Compounds for the Activity of Reducing or Enhancing the Expression of Contactin

The present invention includes methods of screening test compounds for the activity of reducing or enhancing the expression of contactin, including providing at least one cell, contacting said at least one cell with at least one test compound; and measuring the change in contactin mRNA, contactin DNA or contactin protein in the at least one cell. The present invention also includes compositions, including

pharmaceutical compositions, that include at least one test compound identified by these methods.

In operation of this methods, a cell of the present invention is contacted with at least one test compound. The change in the amount of contactin mRNA, contactin DNA or contactin protein in the cell is then measured using at least one method of the present invention. Preferably, the amount of contactin mRNA, contactin DNA or contactin protein exhibited by the cell is known prior to the cell being contacted with a test compound, but that need not be the case. Compounds that increase or decrease the amount of contactin mRNA, contactin DNA or contactin protein in a cell are presumptive therapeutic agents to increase or decrease contactin mRNA, contactin DNA or contactin protein in a cell.

Test compounds that increase the amount of contactin mRNA, contactin DNA or contactin protein in a cell have presumptive therapeutic activity of decreasing cell mobility, enhancing the binding of cells to Ng-CAM, increasing interaction of cells with the extracellular matrix glycoprotein restrictin, and promoting neurite outgrowth. Test compounds that decrease the amount of contactin mRNA, contactin DNA or contactin protein in a cell have presumptive therapeutic activity of treating a neurodegenerative disease such as multiple sclerosis, lateral sclerosis, MELAS or MERRF, increase cell mobility, decreasing the binding of cells to Ng-CAM, decreasing interaction of cells with the extracellular matrix glycoprotein restrictin, and repressing neurite outgrowth. The identified test compounds can be evaluated using the methods set forth herein.

Pharmacology and toxicity of test compounds

The structure of a test compound can be determined or confirmed by methods known in the art, such as mass spectroscopy. For test compounds stored for extended periods of time under a variety of conditions, the structure, activity and potency thereof can be confirmed.

Identified test compounds can be evaluated for a particular activity using are-recognized methods and those disclosed herein. For example, if an identified test

compound is found to have anticancer cell activity in vitro, then the test compound would have presumptive pharmacological properties as a chemotherapeutic to treat cancer. Such nexuses are known in the art for several disease states, and more are expected to be discovered over time. Based on such nexuses, appropriate confirmatory *in vitro* and *in vivo* models of pharmacological activity, and toxicology, and be selected and performed. The methods described herein can also be used to assess pharmacological selectivity and specificity, and toxicity.

Identified test compounds can be evaluated for toxicological effects using known methods (see, Lu, *Basic Toxicology, Fundamentals, Target Organs, and Risk Assessment*, Hemisphere Publishing Corp., Washington (1985); U.S. Patent Nos; 5,196,313 to Culbreth (issued March 23, 1993) and 5,567,952 to Benet (issued October 22, 1996)). For example, toxicology of a test compound can be established by determining in vitro toxicity towards a cell line, such as a mammalian, for example human, cell line. Test compounds can be treated with, for example, tissue extracts, such as preparations of liver, such as microsomal preparations, to determine increased or decreased toxicological properties of the test compound after being metabolized by a whole organism. The results of these types of studies are predictive of toxicological properties of chemical s in animals, such as mammals, including humans.

Alternatively, or in addition to these *in vitro* studies, the toxicological properties of a test compound in an animal model, such as mice, rats, rabbits, dogs or monkeys, can be determined using established methods (see, Lu, supra (1985); and Creasey, *Drug Disposition in Humans, The Basis of Clinical Pharmacology*, Oxford University Press, Oxford (1979)). Depending on the toxicity, target organ, tissue, locus and presumptive mechanism of the test compound, the skilled artisan would not be burdened to determine appropriate doses, LD_{50} values, routes of administration and regimes that would be appropriate to determine the toxicological properties of the test compound. In addition to animal models, human clinical trials can be performed following established procedures, such as those set forth by the United States Food and

Drug Administration (USFDA) or equivalents of other governments. These toxicity studies provide the basis for determining the efficacy of a test compound *in vivo*.

Efficacy of test compounds

Efficacy of a test compound can be established using several art recognized methods, such as in vitro methods, animal models or human clinical trials (see, Creasey, supra (1979)). Recognized *in vitro* models exist for several diseases or conditions. For example, the ability of a test compound to extend the life-span of HIV-infected cells *in vitro* is recognized as an acceptable model to identify chemicals expected to be efficacious to treat HIV infection or AIDS (see, Daluge et al., *Antimicro. Agents Chemother.* 41:1082-1093 (1995)). Furthermore, the ability of cyclosporin A (CsA) to prevent proliferation of T-cells in vitro has been established as an acceptable model to identify chemicals expected to be efficacious as immunosuppressants (see, Suthanthiran et al., *supra* (1996)). For nearly every class of therapeutic, disease or condition, an acceptable *in vitro* or animal model is available. The skilled artisan is armed with a wide variety of such models as they are available in the literature or from the USFDA or the National Institutes of Health (NIH). In addition, these *in vitro* methods can use tissue extracts, such as preparations of liver, such as microsomal preparations, to provide a reliable indication of the effects of metabolism on a test compound.

Similarly, acceptable animal models can be used to establish efficacy of test compounds to treat various diseases or conditions. For example, the rabbit knee is an accepted model for testing agents for efficacy in treating arthritis (see, Shaw and Lacy, J. *Bone Joint Surg. (Br.)* 55:197-205 (1973)). Hydrocortisone, which is approved for use in humans to treat arthritis, is efficacious in this model which confirms the validity of this model (see, McDonough, *Phys. Ther.* 62:835-839 (1982)). When choosing an appropriate model to determine efficacy of test compounds, the skilled artisan can be guided by the state of the art, the USFDA or the NIH to choose an appropriate model, doses and route of administration, regime and endpoint and as such would not be unduly burdened. In addition to animal models, human clinical trials can

be used to determine the efficacy of test compounds. The USFDA, or equivalent governmental agencies, have established procedures for such studies.

Selectivity of test compounds

The *in vitro* and *in vivo* methods described above also establish the selectivity of a candidate modulator. It is recognized that chemicals can modulate a wide variety of biological processes or be selective. Panels of cells as they are known in the art can be used to determine the specificity of the a test compound (WO 98/13353 to Whitney et al., published April 2, 1998). Selectivity is evident, for example, in the field of chemotherapy, where the selectivity of a chemical to be toxic towards cancerous cells, but not towards non-cancerous cells, is obviously desirable. Selective modulators are preferable because they have fewer side effects in the clinical setting. The selectivity of a test compound can be established in vitro by testing the toxicity and effect of a test compound can be established *in vitro* by testing the toxicity and effect of a test compound on a plurality of cell lines that exhibit a variety of cellular pathways and sensitivities. The data obtained form these *in vitro* toxicity studies can be extended to animal model studies, including human clinical trials, to determine toxicity, efficacy and selectivity of a test compound.

The selectivity, specificity and toxicology, as well as the general pharmacology, of a test compound can be often improved by generating additional test compounds based on the structure/property relationship of a test compound originally identified as having activity. Test compounds can be modified to improve various properties, such as affinity, life-time in blood, toxicology, specificity and membrane permeability. Such refined test compounds can be subjected to additional assays as they are known in the art or described herein. Methods for generating and analyzing such compounds or compositions are known in the art, such as U.S. Patent No. 5,574,656 to Agrafiotis et al.

Pharmaceutical compositions

The present invention also encompasses a test compound in a pharmaceutical composition comprising a pharmaceutically acceptable carrier prepared for storage and preferably subsequent administration, which have a pharmaceutically effective amount of the test compound in a pharmaceutically acceptable carrier or diluent. Acceptable carriers or diluents for therapeutic use are well known in the pharmaceutical art, and are described, for example, in Remington's Pharmaceutical Sciences, Mack Publishing Co., (A.R. Gennaro edit. (1985)). Preservatives, stabilizers, dyes and even flavoring agents can be provided in the pharmaceutical composition. For example, sodium benzoate, sorbic acid and esters of p-hydroxybenzoic acid can be added as preservatives. In addition, antioxidants and suspending agents can be used.

The test compounds of the present invention can be formulated and used as tablets, capsules or elixirs for oral administration; suppositories for rectal administration; sterile solutions, suspensions or injectable administration; and the like. Injectables can be prepared in conventional forms either as liquid solutions or suspensions, solid forms suitable for solution or suspension in liquid prior to injection, or as emulsions. Suitable excipients are, for example, water, saline, dextrose, mannitol, lactose, lecithin, albumin, sodium glutamate, cysteine hydrochloride and the like. In addition, if desired, the injectable pharmaceutical compositions can contain minor amounts of nontoxic auxiliary substances, such as wetting agents, pH buffering agents and the like. If desired, absorption enhancing preparation, such as liposomes, can be used.

The pharmaceutically effective amount of a test compound required as a dose will depend on the route of administration, the type of animal or patient being treated, and the physical characteristics of the specific animal under consideration. The dose can be tailored to achieve a desired effect, but will depend on such factors as weight, diet, concurrent medication and other factors which those skilled in the medical arts will recognize. In practicing the methods of the present invention, the pharmaceutical compositions can be used alone or in combination with one another, or in combination with other therapeutic or diagnostic agents. These products can be

utilized *in vivo*, preferably in a mammalian patient, preferably in a human, or *in vitro*. In employing them *in vivo*, the pharmaceutical compositions can be administered to the patient in a variety of ways, including parenterally, intravenously, subcutaneously, intramuscularly, colonically, rectally, nasally or intraperiotoneally, employing a variety of dosage forms. Such methods can also be used in testing the activity of test compounds *in vivo*.

As will be readily apparent to one skilled in the art, the useful in vivo dosage to be administered and the particular mode of administration will vary depending upon the age, weight and type of patient being treated, the particular pharmaceutical composition employed, and the specific use for which the pharmaceutical composition is employed. The determination of effective dosage levels, that is the dose levels necessary to achieve the desired result, can be accomplished by one skilled in the art using routine methods as discussed above, and can be guided by agencies such as the USFDA or NIH. Typically, human clinical applications of products are commenced at lower dosage levels, with dosage level being increased until the desired effect is achieved. Alternatively, acceptable *in vitro* studies can be used to establish useful doses and routes of administration of the test compounds.

In non-human animal studies, applications of the pharmaceutical compositions are commenced at higher dose levels, with the dosage being decreased until the desired effect is no longer achieved or adverse side effects are reduced of disappear. The dosage for the test compounds of the present invention can range broadly depending upon the desired affects, the therapeutic indication, route of administration and purity and activity of the test compound. Typically, dosages can be between about 1 ng/kg and about 10 ng/kg, preferably between about 10 ng/kg and about 1 mg/kg, more preferably between about 100 ng/kg and about 100 micrograms/kg, and most preferably between about 1 microgram/kg and about 10 micrograms/kg.

The exact formulation, route of administration and dosage can be chosen by the individual physician in view of the patient's condition (see, Fingle et al., in *The*

Pharmacological Basis of Therapeutics (1975)). It should be noted that the attending physician would know how to and when to terminate, interrupt or adjust administration due to toxicity, organ dysfunction or other adverse effects. Conversely, the attending physician would also know to adjust treatment to higher levels if the clinical response were not adequate. The magnitude of an administrated does in the management of the disorder of interest will vary with the severity of the condition to be treated and to the route of administration. The severity of the condition may, for example, be evaluated, in part, by standard prognostic evaluation methods. Further, the dose and perhaps dose frequency, will also vary according to the age, body weight and response of the individual patient, including those for veterinary applications.

Depending on the specific conditions being treated, such pharmaceutical compositions can be formulated and administered systemically or locally. Techniques for formation and administration can be found in Remington's Pharmaceutical Sciences, 18th Ed., Mack Publishing Co., Easton, PA (1990). Suitable routes of administration can include oral, rectal, transdermal, otic, ocular, vaginal, transmucosal or intestinal administration; parenteral delivery, including intramuscular, subcutaneous, intramedullary injections, as well as intrathecal, direct intraventricular, intravenous, intraperitoneal, intranasal, or intraocular injections.

For injection, the pharmaceutical compositions of the present invention can be formulated in aqueous solutions, preferably in physiologically compatible buffers such as Hanks' solution, Ringer's solution or physiological saline buffer. For such transmucosal administration, penetrans appropriate to the barrier to be permeated are used in the formulation. Such penetrans are generally known in the art. Use of pharmaceutically acceptable carriers to formulate the pharmaceutical compositions herein disclosed for the practice of the invention into dosages suitable for systemic administration is within the scope of the invention. With proper choice of carrier and suitable manufacturing practice, the compositions of the present invention, in particular, those formulation as solutions, can be administered parenterally, such as by intravenous injection. The pharmaceutical compositions can be formulated readily using

pharmaceutically acceptable carriers well known in the art into dosages suitable for oral administrations. Such carriers enable the test compounds of the invention to be formulated as tables, pills, capsules, liquids, gels, syrups, slurries, suspensions and the like, for oral ingestion by a patient to be treated.

Agents intended to be administered intracellularly may be administered using techniques well known to those of ordinary skill in the art. For example, such agents may be encapsulated into liposomes, then administered as described above. Substantially all molecules present in an aqueous solution at the time of liposome formation are incorporated into or within the liposomes thus formed. The liposomal contents are both protected from the external micro-environment and, because liposomes fuse will cell membranes, are efficiently delivered into the cell cytoplasm. Additionally, due to their hydrophobicity, small organic molecules can be directly administered intracellularly.

Pharmaceutical compositions suitable for use in the present invention include compositions wherein the active ingredients are contained in an effective amount to achieve its intended purpose. Determination of the effective amount of a pharmaceutical composition is well within the capability of those skilled in the art, especially in light of the detailed disclosure provided herein. In addition to the active ingredients, these pharmaceutical compositions can contain suitable pharmaceutically acceptable carriers comprising excipients and auxiliaries which facilitate processing of the active chemicals into preparations which can be used pharmaceutically. The preparations formulated for oral administration may be in the form of tables, dragees, capsules or solutions. The pharmaceutical compositions of the present invention can be manufactured in a manner that is itself known, for example by means of conventional mixing, dissolving, granulating, dragee-making, emulsifying, encapsulating, entrapping or lyophilizing processes. Pharmaceutical formulations for parenteral administration include aqueous solutions of active chemicals in water-soluble form.

Additionally, suspensions of the active chemicals may be prepared as appropriate oily injection suspensions. Suitable lipophilic solvents or vehicles include

fatty oils such as sesame oil, or synthetic fatty acid esters, such as ethyl oleate or triglycerides or liposomes. Aqueous injection suspensions may contain substances what increase the viscosity of the suspension, such as sodium carboxymethyl cellulose, sorbitol or dextran. Optionally, the suspension can also contain suitable stabilizers or agents that increase the solubility of the chemicals to allow for the preparation of highly concentrated solutions.

Pharmaceutical compositions for oral use can be obtained by combining the active chemicals with solid excipient, optionally grinding a resulting mixture, and processing the mixture of granules, after adding suitable auxiliaries, if desired, to obtain tables or dragee cores. Suitable excipients are, in particular, fillers such as sugars, including lactose, sucrose, mannitol or sorbitol; cellulose preparations such as, for example, maize starch, wheat starch, rice starch, potato starch, gelatin, gum tragacanth, methyl cellulose, hydroxypropylmethyl-cellulose, sodium carboxymethylcellulose and/or polyvinylpyrrolidone. If desired, disintegrating agents can be added, such as the cross-linked polyvinyl pyrolidone, agar, alginic acid or a salt thereof such as sodium alginate. Dragee cores can be provided with suitable coatings. Dyes or pigments can be added to the tablets or dragee coatings for identification or to characterize different combinations of active doses.

The test compounds of the present invention, and pharmaceutical compositions that include such test compounds are useful for treating a variety of ailments in a patient, including a human. As set forth in the Examples, the test compounds of the present invention have antibacterial, antimicrobial, antiviral, anticancer cell, antitumor and cytotoxic activity. A patient in need of such treatment can be provided a test compound of the present invention, preferably in a pharmacological composition in an effective amount to reduce the number or growth rate of bacteria, microbes, cancer cells or tumor cells in said patent, or to reduce the infectivity of viruses in said patient. The amount, dosage, route of administration, regime and endpoint can all be determined using the procedures described herein or by

appropriate government agencies, such as the United Stated Food and Drug Administration.

Methods of Treating a Neurodegenerative Disease

The present invention also includes methods of treating a neurodegenerative disease using the compositions or compounds identified by the methods of the present invention. As set forth herein. compounds that increase or decrease the amount of contactin mRNA, contactin DNA or contactin protein in a cell have presumptive therapeutic activities. These therapeutic activities can be confirmed using the methods of the present invention. Preferably, this aspect of the invention includes administering an effective amount of a pharmaceutical composition of the present invention by a route of administration sufficient to provide a treatment that is remedial, therapeutic, palliative, rehabilitative, preventative, impeditive or prophylactic in nature using appropriate endpoints for such treatments.

Appropriate end-points or parameters for the treatment of multiple sclerosis include: a reduction in the progression of the disorder or the frequency or occurrence of the symptoms characteristic of the disorder, such as, for example, cognitive impairment, optic neritis, diplopia, vertigo, weakness, tremor, spasticity, hystagmus, ataxia, visual loss, piplopia, fatigue, sphincter dysfunction, gait impairment, or the amount of immunoglobulins in the cerebral spinal fluid. Appropriate end-points or parameters for the treatment of lateral sclerosis include: a reduction in the progression of the disorder or the frequency or occurrence of the symptoms characteristic of the disorder, such as, for example, the loss of function of upper or lower neurons resulting in progressive skeletal muscular wasting or weakness. Appropriate end-points or parameters for the treatment of MELAS include: a reduction in the progression of the disorder or the frequency or occurrence of the symptoms characteristic of the disorder, such as, for example, focal or generalized seizures, dementia, headaches, vomiting, hemianopsia, cortical blindness, hearing loss, elevated serum lactate or encephalopathy. Appropriate end-points or parameters for the treatment of MERRF include: a reduction in the progression of the disorder or the

frequency or occurrence of the symptoms characteristic of the disorder, such as, for example, seizures, ataxia, lactic acidosis, dysarthia, optic atrophy, hearing loss, dementia, nystagmus, spasticity, muscular weakness or increased serum pyruvate

Another aspect of the present invention is a method of treatment for a neurodegenerative disease, including: providing a sample from a patient having said neurodegenerative disease; measuring the amount of contactin protein, contactin DNA or contactin mRNA present in the sample; and administering to the patient an effective amount of a composition of the present invention, wherein a positive correlation exists between the amount of contactin protein or contactin mRNA present in a sample from a patient and the efficacy of said compound. This aspect of the present invention uses the methods described herein to obtain samples from a patient having a neurodegenerative disease and measuring the amount of contactin mRNA, contactin DNA or contactin protein in the sample. If the sample exhibits an increased or decreased level of contactin mRNA, contactin DNA or contactin protein, an appropriate composition, such as a pharmaceutical composition of the present invention, is administered to the patient to modulate the amount of contactin mRNA, contactin DNA or contactin protein towards normal levels. The pharmaceutical composition can be administered in an effective amount by an appropriate route of administration using an appropriate regime. The effectiveness of such treatment can be measured using end-points or parameters appropriate for the neurodegenerative disease being treated. Preferably, such treatments are remedial, therapeutic, palliative, rehabilitative, preventative, impeditive or prophylactic in nature.

Methods for Identifying Pharmaceutical Targets and Pharmaceutical Targets Identified by Such Methods

The present invention includes methods for identifying pharmaceutical targets for compounds that modulate contactin expression or activity and targets identified by methods of the present invention. The present invention also includes pharmaceutical targets identified by such methods.

Methods for identifying a pharmaceutical target include providing a compound that modulates contactin expression or activity, such as the amount of contactin mRNA, contactin DNA or contactin protein in a cell; and identifying the cellular component that binds with the compound. In practice, the method includes providing a cell, such as a cell of the present invention, that expresses an increased, decreased or normal amount of contactin mRNA, contactin DNA or contactin protein. Alternatively, an extract of the cell can be provided. The cell or extract thereof is then contacted with a compound that modulates the amount of contactin mRNA, contactin DNA or contactin protein in a sample. The binding of the compound to a cellular component is then detected. The cellular component that binds with the compound is a presumptive therapeutic target for the treatment of neurodegenerative diseases, such as multiple sclerosis, lateral sclerosis, MELAS or MERRF.

The binding of compound to a cellular component can be detected using a variety of methods. For example, the compound can be attached to a detectable label such that the location of the compound can be monitored in a cell. The compound-cellular component can then be isolated using affinity chromatography or immunoprecipitation methods that use antibodies specific for the compound or label. Preferably, gel-shift assays can be used to detect compound-cellular component binding. For example, a compound attached to a detectable label has a certain mobility though a gel in response to an electrical current due to a variety of parameters, including the molecular weight of the compound attached to a detectable label. The binding of compound to cellular component changes the mobility of the resulting compound-cellular component complex through the gel due to the increased molecular weight of the complex relative to the cellular component and compound alone. The regions of the gel containing the compound-cellular component can be collected, and the cellular component identified using methods known in the art, such as reactivity with antibodies, molecular weight, localization of the cellular component in a cell, and activity of the cellular component.

In addition, the ability of a compound to modulate signal transduction pathways can be determined. The ability of a compound to modulate an identified signal transduction pathways identifies such signal transduction pathway as a therapeutic target. A variety of cells that comprise reporter genes that report an increased or decreased activity of a signal transduction pathway in response to a compound are known in the art. Such cells can also be made using methods known in the art (see, WO 98/13353 to Whitney, published April 2, 1999; U.S. Patent No. 5,298,429 to Evans et al., issued March 29, 1994; and Skarnes et al., Genes and Development 6:903-918 (1992)). Compounds of the present invention can be contacted with such cells and the expression of the reporter gene monitored to identify signal transduction pathways modulated by the compound. Such identified signal transduction pathways are themselves pharmaceutical targets, as are the individual components of the identified signal transduction pathway.

The following examples are offered by way of illustration, and not by way of limitation.

EXAMPLES

EXAMPLE 1

AMPLIFICATION OF CONTACTIN MRNA

This example establishes that the methods used to detect contactin mRNA are specific for contactin mRNA.

Preparations of cDNA having nucleic acids encoding contactin were prepared from total RNA from human brain tissue purchased from Clontech (Palo Alto, CA; catalogue No: 64020-1). cDNA preparations were made using Superscript II kits from GIBCO using oligo dT as a primer.

Contactin nucleic acids in samples was measured by Real-Time PCR amplification methods and SYBR® Green detection system using the primers:

5'-TCAGTAAGGTCTGGTTCACGCTAT-3' (SEQ ID NO:1),

5'-TCCCGTCACTGTAGATTCATTTGA-3' (SEQ ID NO:2),

5'-CCCCAAGTCTTCTCGGCTTA-3' (SEQ ID NO:3),

5'-CAACACATTCAGAATTCCAAGTAGACA-3' (SEQ ID NO:4),

5'-TCCCCAAGTCTTCTCGGCTTA-3' (SEQ ID NO:5),

5'-CCCATCCCAGCTCAGAAGAC-3' (SEQ ID NO:6), and

5'-GCCGCAGAAATTGGAAGG-3' (SEQ ID NO:7).

SEQ ID NO: 1, SEQ ID NO:3, SEQ ID NO:5 and SEQ ID NO:6 are forward primers. SEQ ID NO:2, SEQ ID NO:4 and SEQ ID NO:7 are reverse primers. Preferred primer pairs were SEQ ID NO:6 and SEQ ID NO:7; SEQ ID NO:1 and SEQ ID NO:2; SEQ ID NO:5 and SEQ ID NO:4; and SEQ ID NO:3 and SEQ ID NO:4.

The PCR amplification products were separated using 4% agarose gels that included appropriate molecular weight markers. The gels were stained with ethidium bromide and all showed a single band of the correct predicted size for contactin-derived PCR products. Thus, these results establish that the primer pairs specifically amplify contactin mRNA.

EXAMPLE 2

OPTIMIZATION OF QUANTITATIVE REAL TIME PCR

The use of real time PCR to quantitate levels of specific nucleic acids has been described in the art (see Freeman et al., BioTechniques 26:112-125 (1999), for a recent review as well as Heid et al., Genome Research 6:986-994 (1996); Gibson et al., Genome Research 6:995-1001 (1996), U.S. Patent No., 4,683,202; U.S. Patent No. 4,683,195; U.S. Patent No. 4,965,188; U.S. Patent No. 5,035,996; SYBR® Green PCR and RT-PCR Reagents, Protocol, Applied Biosystems, 1998; and Spiess et al., BioTechniques 26:46-50 (1990, all references being hereby incorporated by reference).

For ease of understanding, a brief explanation of quantitative real time PCR (Q-RTPCR) follows.

Until recently, the traditional means of measuring the products of a specific PCR reaction was the "end-point" method of analysis, in which the reaction products are measured and quantitated after the amplification reactions are completed. In contrast, "real-time" PCR monitors amplification reactions in the thermal cycler as they progress. Q-RTPCR provides for improved quantification, because quantification is achieved most accurately during the linear range of amplification, and more information about the amplification reactions is obtained for each cycle.

For example, the normalized (i.e., to a passive reference dye that does not bind DNA) fluorescence intensity ("ΔR_n"), which indicates the magnitude of the signal generated by a given set of PCR conditions, can be measured during each cycle. From such data, the cycle at which a statistically significant increase in ΔR_n is first detected can be determined. The "threshold cycle" or "C_T value" is determined at one log above the signal first detected and provides a quantitative measure of the amount of the input nucleic acid template of interest present in the original sample.

In order to optimize the PCR reactions for use with the SYBR® Green detection system, which does not discriminate between different amplification products, different concentrations of different primers were used to normalize the rates of PCR product amplification. The results are shown in Table 1.

Preferred primer pairs and concentrations reflect those instances where the average C_T is relatively low (i.e., sensitivity is high) and the average ΔR_n is relatively high (i.e., the range of magnitude of signal is large). Based on these criteria, preferred primer pairs and concentrations include:

SEQ ID NO:3 (900 nM) and SEQ ID NO:4 (900 nM),

SEQ ID NO:1 (300 nM) and SEQ ID NO:2 (300 nM),

SEQ ID NO:1 (900 nM) and SEQ ID NO:2 (900 nM), and

SEQ ID NO:6 (300 nM) and SEQ ID NO:7 (900 nM).

In order to correct for sample-to-sample variation, an internal RNA normalizer is used in Q-RTPCR. The RNA normalizer may be an endogenous RNA species such as, e.g., an mRNA encoding a constitutively-expressed protein like actin or glyceraldehyde-3-phosphate dehydrogenase (GAPDH), or a ribosomal RNA such as 18S or 28S rRNA; RNA molecules produced *in vitro* may also be used as normalizers. Results of Q-RTPCR analyses are thus often expressed as relative amounts.

For example, when the normalizer is actin and the nucleic acid that is being quantitated is a nucleic acid encoding contactin, the relative amount of contactin RNA is determined as compared to the normalizer actin according to standard curves created for both gene sequences for each RNA sample. Standard curves were typically prepared using between about three and four different amounts of input RNA in triplicate reactions. Standard curves are plotted as log input concentration (x axis) versus C_t (y axis, also log scale). For each standard curve, the slope (m) and the y-intercept (b) are calculated using standard analysis software.

The log input amount for the normalizer (nN) is calculated for a given C_T (C_T^0). For example, when $C_T^0 = 20$,

$$nN = \frac{(20 - b_N)}{m_n}$$

For a specific target (T) sequence of interest, C_T^T (the C_T required to reach a log input amount equal to nN) is determined by the formula

$$C_T^T = (m_T \text{ x } nN) + b_T$$

The normalized target C_t (normalized C_t^T) is calculated according to the formula:

$$\text{normalized } C_T{}^T = C_T{}^T - C_T{}^0$$

In the present example, the efficiency of these PCR reactions was compared to the efficiencies for control reactions using primer pairs for actin. Standard curves for determining changes in Ct for these reactions were determined by plotting the concentration of primers used vs. the signal from the Taqman SYBR® Green detection system for contactin as compared to the results obtained for actin (Table 2). The slope of the contactin plots were subtracted from the results for the actin plots. An absolute difference of less than about 0.1 is considered a preferable assay. The contactin primer pair SEQ ID NO:1 and SEQ ID NO:2 were preferred using actin primers as a control. The preferred actin primer pairs were 5'-CTGGAACGGTGAAGGTGACA-3' (SEQ ID NO:8) forward primer and 5'-CGGCCACATTGTGAACTTTG-3' (SEQ ID NO:9) reverse primer. These conditions resulted in an absolute difference of 0.063.

TABLE 1

NORMALIZATION OF PCR AMPLIFICATION RATES USING CONTACTIN PRIMERS

Forward Primer		Reverse Primer		Average CT	Average Rn
SEQ ID NO:	Concentration (nM)	SEQ ID NO:	Concentration (nM)		
3	100	4	100	4.35	19.43
3	300	4	100	4.77	19.36
3	900	4	100	4.49	19.39
3	100	4	300	4.26	19.35
3	300	4	300	5.46	19.16
3	900	4	300	6.17	19.15
3	100	4	900	4.42	19.20
3	300	4	900	6.08	18.89
3	900	4	900	6.82	18.65
1	100	2	100	4.61	19.24

Forward Primer		Reverse Primer		Average CT	Average Rn
SEQ ID NO:	Concentration (nM)	SEQ ID NO:	Concentration (nM)		
1	300	2	100	4.69	19.19
1	900	2	100	4.68	19.21
1	100	2	300	4.99	19.04
1	300	2	300	6.09	18.75
1	900	2	300	5.85	19.14
1	100	2	900	4.75	19.15
1	300	2	900	6.09	18.90
1	900	2	900	6.20	18.57
6	100	7	100	4.44	19.26
6	300	7	100	4.55	18.76
6	900	7	100	4.17	19.05
6	100	7	300	4.99	19.01
6	300	7	300	6.16	18.82
6	900	7	300	6.28	18.84
6	100	7	900	4.50	19.08
6	300	7	900	6.44	18.68
6	900	7	900	6.08	18.95

TABLE 2

COMPARISON OF EFFICIENCIES OF PCR REACTIONS FOR CONTACTIN AND ACTIN

Target	Primer Pairs (Forward / Reverse) (SEQ ID NO:)	Slope	Difference of Slope for Contactin and Actin
Actin	8 / 9	-3.322	Not Applicable
Contactin	3 / 4	-1.854	1.468
Contactin	1 / 2	-3.259	0.063
Contactin	6 / 7	-2.942	0.380

EXAMPLE 3

EXPRESSION OF CONTACTIN mRNA IN NEUROLOGICAL SAMPLES

Human brain samples were obtained from the occipital pole post mortem as soon as possible after death. Samples were obtained from a patient diagnosed with multiple sclerosis (MS) and a control patient. The biological samples were frozen and stored at -80°C, and shipped on dry ice to analysis facilities.

Total RNA was isolated from the biological samples described in Example 2 using the TRIzol® Reagent supplied by Life Technologies™ following the manufacturer's instructions (one version of the TRIzol® Reagent is described in U.S. Patent No. 5,346,994). Briefly, tissue samples were homogenized in TRIzol® Reagent (1 mL of TRIzol® Reagent per 50 mg to 100 mg of tissue). The homogenized samples were incubated at about 15°C to 30°C for about five minutes. Chloroform (0.2 ml per ml of TRIzol® Reagent used in the initial homogenization) were added and the mixture shaken vigorously for fifteen seconds, incubated at about 15°C to 30°C for about two or three minutes, and centrifuged at about 12,000 x g for fifteen minutes at about 2°C to 8°C. The aqueous phase was transferred to a fresh container and the RNA therein was precipitated by mixing with 0.5 ml of isopropyl alcohol per 1 ml of TRIzol® Reagent used in the initial homogenization. The mixture was incubated at about 15°C to 30°C for about ten minutes and centrifuged at about 12,000 x g for ten minutes at about 2°C to 8°C. The supernatant was removed, and the resulting pellet was washed once with 75% ethanol and recollected by centrifugation at about 12,000 x g for five minutes at about 2°C to 8°C. The resulting pellet was collected and dried.

The contactin mRNA and actin mRNA in the pellets were amplified and quantitated as described in Examples 1 and 2. The percent expression of contactin mRNA was calculated as [(contactin mRNA / actin mRNA) x 100]. For the control sample, the percent expression of contactin mRNA was 22.84. For the multiple sclerosis sample, the percent expression of contactin mRNA was 95.22. These results

establish that samples of neurological tissues from patients being diagnosed with MS have elevated levels of contactin mRNA.

EXAMPLE 4

ASSAYS OF CONTACTIN PROTEIN AND MRNAS IN BIOLOGICAL SAMPLES

Biological samples comprising material from central nervous system tissues, lung tissues, kidney tissues, skeletal muscle, epithelium, blood or amniotic fluid are prepared according to known methods from patients diagnosed as, or suspected of, having MS, ALS, MELAS, MERRF and/or other neurological disorders. Control samples are taken from individuals, preferably from age- and gender-matched or genetically related individuals, who are apparently free of such neurological disorders. Biological samples may be from living or dead individuals. The biological samples are evaluated for levels of contactin proteins and/or contacting-encoding mRNAs.

To detect and quantitate levels of contactin proteins, biological samples are stained using immunohistochemical methods, or evaluated by immunoassay, using an antibody specific for contactin. Such antibodies include, for example, the monoclonal antibody Neuro-1 (Reid et al., Molecular Brain Research 21:1-8 (1994)) and those described by Brummendorf et al. (Neuron 10:711-727, (1993)). The methods described herein in the Detailed Description and Examples 1 through 3 are used to detect and quantitate levels of contactin-encoding mRNA,

Consistent with the results obtained in Example 3, the amount of contactin in the samples from patients with MS, ALS, MELAS, MERRF and/or other neurological disorders are altered relative to the amount of contactin in the control samples.

EXAMPLE 5

SCREENING OF TEST COMPOUNDS FOR INCREASING OR DECREASING EXPRESSION OF CONTACTIN MRNA OR CONTACTIN PROTEINS

Cells that express normal, increased and decreased amounts of contactin mRNA or contactin protein are provided. Such cells may contain, for example, an expression construct that comprises a nucleic acid encoding a contactin protein (Reid et al., Molecular Brain Research 21:1-8 (1994), Ogawa et al., Neurosci. Lett. 218:173-176 (1996)). When the contactin-encoding nucleic acid is transcribed in the "sense" orientation, increased amounts of the contactin mRNA and protein are expected to result. In contrast, when the contactin-encoding nucleic acid is transcribed in the "antisense" orientation, decreased amounts of the contactin mRNA and protein are expected to result.

These cells are contacted with the individual members of a library of test compounds known to have pharmaceutical activity. The amount of contactin mRNA and contactin protein are measured in such cells before and after being contacted with the test compounds using the methods of the present invention. Test compounds that increase or decrease the amount of contactin mRNA or contactin protein are identified and are presumptive therapeutic agents to increase or decrease the amount of contactin mRNA or contactin protein in a patient.

EXAMPLE 6

DIFFERENTIAL GENE EXPRESSION IN AD BRAIN SAMPLES

Human brain tissue from the occipital pole of individuals diagnosed with Alzheimer's disease (AD samples), with MS or from control individuals (control or "C" samples) was promptly obtained post mortem and stored frozen at -80°C. Total poly A+ RNA was isolated from frozen brain tissue after thawing and extracting the tissue according to standard protocols. Poly (A)+ RNAs were prepared from 3 different regions of the brain from autopsies of 5 normal (control) human and 7 Alzheimer's diseased patients. Preparations of radiolabeled cDNAs derived from the poly (A)+

RNAs were prepared using reactions of the Superscript II kit. using oligo-dT as a primer, essentially according to the manufacturer's (Life Technologies) protocol in order to produce a set of labeled probes. The labeled probes were hybridized with Genome Systems, Inc. (St. Louis. MO) GDA 1.3 (Gene Discovery Array) membranes.

(The choice of assay systems for gene discovery can be varied, for example. to include filter systems from Genome Systems, Inc. (GDA). Research Genetics, Inc. or Clontech Laboratories, Inc. (Palo Alto, CA), or high-density microarrays such as Affymetrix GeneChip and Genome Systems/Incyte/Synteni (GEM). For example, the GeneChip containing 40.000 oligonucleotides representing 40,000 human genes (35,000 ESTs and ~6.000 full-length cDNAs) from Affymetrix, Inc. or the GEM chip containing 7,000 cDNAs representing 7,000 ESTs with ~4,000 known human genes from GenomeSystems Inc., may optionally be screened as high throughput microarrays.)

The probed membranes were washed and exposed to Phosphorimager screens in order to produce a series of digital image files showing the intensities of hybridization of the probes to individual positions on the membrane. According to the manufacturer (Genome Systems, Inc., St. Louis, MO), each individual position on the filter has a DNA sequence derived from a specific known gene. The manufacturer's identification of the gene from which the DNA sequence present at a given position is derived is generally accurate but. in a few instances described herein, some variation was seen.

The image files containing 18,000 differential brain gene expression profile of normal and Alzheimer's patients were initially processed (at Genome Systems, Inc.) for background normalization and average values. These results were used to rank the degree of labeled probe hybridization at each position, both in terms of genetic sequences that are present in greater amounts in AD patients as compared to control samples (*i.e.*, "up-regulated" genes in AD), as well those present in lower amounts in AD patients as compared to control samples (*i.e.*, "down-regulated" genes in AD).

The ranked data were further analyzed as described herein using, *inter alia*, algorithms to analyze gene clusters that are altered in the diseased brains with special regard to changed gene expression profiles in several affected regions of the brains. Such algorithms are disclosed in U.S. Patent Application Serial No. 09/397,380, filed September 15, 1999, the contents of which are hereby incorporated by reference. The results are shown in Table 3 and summarized in Table 4A. Twenty-eight genes were down-regulated greater than 5-fold, and 38 genes were up-regulated greater than 5-fold, in Alzheimer's diseased (AD) brains compared to normal, control (C) brains.

Table 4A

Differential Gene Expression in Neurodegenerative Diseased Brain

Disease	**Protein Encoded by DNA Sequence with Altered Expression**	**Change in Expression (Fold Increase or Decrease in Disease/ Brain Region)**
MS	Contactin	Increased
AD	FREAC-2	Increased (MFC 2.1X, ITC 2.1X)
AD	APCL	Increased (MFC 4.3X)
AD	LAP	Increased (ITC 3.6X, OP 2.9X)
AD	Brain-expressed novel protein (EFHD homologue)	Increased (MFC 2.1X)
AD	COX7C	Decreased (OP 0.5X)
AD	PAF	Decreased (ITC 0.5X)
AD	6PTS1	Decreased (ITC 0.4X; OP 0.6X)
AD	VDAC1	Decreased (OP 0.5X)

Disease	Protein Encoded by DNA Sequence with Altered Expression	Change in Expression (Fold Increase or Decrease in Disease/ Brain Region)
AD	UNK-Br40 Brain-expressed novel sequence	Increased (MFC 2.9X)
AD	UNK-Br42 Brain-expressed novel protein	Decreased (OP 0.4X)

Table 4B

Nucleotide Sequences of Oligonucleotide Primers and Probes for Studies of Gene Expression in AD Brain

Forward Primer Designation	Forward Primer Nucleotide Sequence (5' → 3 ')	Reverse Primer Designation	Reverse Primer Nucleotide Sequence (5' → 3 ')	TaqMan Probe Designation	TaqMan Probe Nucleotide Sequence (5' → 3 ')
GS COX7c-143F	GGTCCGTAGGAGCCACTATGAG	GS COX7c-212R	CGACCACTTGTTTTCCACTGAA	GS COX7c-166T	TGGCAAATTCTTCCCAGGGC-CCA
GS VDAC1-319F	CTATAAGCTTGGAAGTGTCT-GTGTGAT	GS VDAC1-415R	AACAAATTGAGTGGTGAGAATA-CAACA	GS VDAC1-352T	CACATGGTGACAACACTCAG-AATCTAAATTGGAC
GS FREAC-2-200F	CCTCTTCTTGTTGCCTAGCT-GAGT	GS FREAC-2-325R	AAACCACTTTCTTAAACTAGAC-ACATTGTT	GS FREAC-2-239T	CCCAGACACTACATTTGGAT-ACAGGTGCCA
GS LAP-183F	AATGCACAATTTCTGGACATG	GS LAP-251R	CCAGACGGTCTCCAGTGTCAG	GS LAP-205T	TCTGTAAGCCCTGTCTCGTT-GGCCA
GS PAF-1-257F	AAGATACCATTTATGAATCC-TTTATAAGGAAT	GS PAF-1-347R	GTTTCCAGTGATTAGATTTCAG-TCATG	GS PAF-1-290T	TGCTCCTAGCCACTGTCTTC-TCCTTTCCAG
GS EFHD-380F	TTATTTGGCCATCTCCTGGT-TATAT	GS EFHD-472R	CAGTAGTTGGAAATGAGTTATT-TTGCA	GS EFHD-406T	CAAATGTGACCCGTGATAAT-GTGATTGAACA
GS 6PTS1-209F	GAATATTAAGAGGTCAACAC-GTGATTG	GS 6PTS1-355R	CTCTCTAAATGATTTTCAGGAT-GTATATTACAAG	GS 6PTS1c-245T	TTTCAATAAATAGGCACTCC-AGAGCACAATGTG
GS APCL-307F	CCATCGAGCCGCAGATCT	GS APCL-375R	GGCGGTACTCCTCATCAAAGG	GS APCL-326T	CCAGGCCACCTGTGCTGTTA-TGAAGC
GS #40-265F	TCATCCCAGGCTATCTCAGA-AGTCT	GS#40-355R	TGGGATCCAATTTGTAGCTTCCT	GS #40-297T	AGGCCTAGAAGGTTGCTGGG-CTCTCTG
GS #42-357F	TCATCTCCTCCATGAATATT-TCCA	GS #42-432R	GCCATACCCAATAGGCAGATCA	GS #42-382T	ATCATCTCCATCTCCACCCA-TCCCATC

Table 5
Relative VDAC1 mRNA Levels [ΔCt value (VDAC1-Actin)] in Human AD Brain and Other Neurological Diseases

Diagnosis (*)	Patient Diagnosis	Temporal Parietal Cortex ΔCt Ave	SD	Cerebellum ΔCt Ave	SD	ΔΔCt (TPC-Cere)	Fold Change 2^-ΔΔCt	(Mean)	B.
A98-01/sAD (Braak stage VI)	sAD #1	4.205	0.332	3.718	0.143	0.487	0.714		
A98-07/AD (Braak stage VI and DLB)	sAD #2	3.658	0.313	3.324	0.152	0.333	0.794		
A97-7/sAD (Braak stage VI)	sAD #3	4.167	0.094	4.518	0.139	-0.352	1.276		
A97-9/sAD (Braak stage VI and DLB)	sAD #4	5.105	0.127	4.25	0.392	0.855	0.553		
A96-8/sAD (Braak stage VI)	sAD #5	4.393	0.262	3.779	0.240	0.613	0.654		
	Ave + SD	***4.305***	***0.523***	***3.918***	***0.470***			**0.798**	**0.281**
A98-18/AD (V)	mAD #1	3.484	0.314	3.216	0.194	0.268	0.830		
A96-25/AD (IV)	mAD #2	3.992	0.055	4.278	0.246	-0.286	1.219		
A97-05/mAD (V)	mAD #3	5.067	0.221	3.466	0.158	1.601	0.330		
A97-10/sAD (V and DLB)	mAD #4	4.056	0.360	3.085	0.503	0.971	0.510		
A96-23/AD III and VaD and DLB	mAD #5	4.069	0.190	2.943	0.099	1.127	0.458		
A98-04/limbic AD III	mAD #6	3.828	0.182	3.860	0.134	-0.032	1.022		
	Ave + SD	***4.083***	***0.529***	***3.475***	***0.509***			**0.728**	**0.351**
A96-11/VaD (no AD change)	No AD #1	4.417	0.157	3.746	0.149	0.671	0.628		
A97-11/mixed (corticobasal degeneration and PD)	No AD #2	3.742	0.033	3.757	0.289	-0.015	1.010		
A97-18/VaD (no AD changes)	No AD #3	3.561	0.097	3.656	0.262	-0.095	1.068		
A97-19/VaD (no AD changes)	No AD #4	4.023	0.039	3.852	0.205	0.171	0.888		
A96-6/DLB (no AD)	No AD #5	5.448	0.276	3.673	0.220	1.775	0.292		

Diagnosis (*)	Patient Diagnosis	Temporal Parietal Cortex ΔCt Ave	SD	Cerebellum ΔCt Ave	SD	ΔΔCt (TPC-Cere)	Fold Change 2^-ΔΔCt	(Mean)	B.
A96-12/DLB (no AD)	No AD #6	4.158	0.185	4.129	0.128	0.028	0.981		
A95-48/DLB (no AD)	No AD #7	3.955	0.127	4.107	0.098	-0.152	1.111		
	Ave + SD	***4.186***	***0.621***	***3.845***	***0.197***			**0.854**	**0.294**
	ΔΔCt(mAD-No AD)	-0.103		-0.371					
	2^-ΔΔCt	**1.074**		**1.293**					
	ΔΔCt(sAD-NoAD)	0.119		0.073					

TABLE 3
Down-Regulated and Up-Regulated Genes in Inferior Temporal Cortex (ITC), Medial Frontal Cortex (MFC) and Occipital Pole (OP) Regions of Alzheimer's Disease (AD) Brains

[TABLE 3A: "↓ ITC" = DOWN-REGULATED GENES IN ITC REGION OF AD BRAIN]

POSITION	↓ITC (AD/C)	MFC (AD/C)	OP (AD/C)	GENE GBACCESSION#
6_h08_8	>3.8↓ 324+654 /2459+3162+ 4267	1635+2359 /1703	931+1538+ 1535 /1867	**COX VIIc** W72273
3_i05_3	>13↓ 0+79 /1607+1534+9 92	961+555 /616	496+2188+405 /670	**Aldolase B** M15656
6_p20_8	>7↓ 81+0 /603+1030+14 66	↑ 1010+2603 /499	945+883+1067 /1109	**Ornithine Aminotransferase** W81709
3_l01_2	>2.7↓ 372+243+1759 /1004+2403	1373+1524 /2282	958+1228+7 /1597	**Thymidine kinase 2** T89262
6_h11_8	>1.8↓ 1001+2525+45 64 /4757+7562	1519+5056 /1904	2908+3045+2356 /4109	**Neuronal tissue-enriched acidic protein (NAP-22); *Gallus gallus* brain alpha-tubulin** W61134
6_n24_8	>2.6↓ 1310+2324 /5988+6416+6 573	2555+2244 /3274	↓ 995+1763+1610 /8937	Homologous to Na channel I protein W81096
4_d19_7	(>∞x↓) 0+0 /973+1241+22 31	1156+0 /156	1243+1943+340 /2208	Homologous to Kruppel-like zinc finger protein Zf9 AA005390
6_f13_2	(>∞x↓) 0+0 /16985+1108+ 948	?↑ 1997+348 /212	3276+0+147 /1809	Homologous to RING/leucine zipper protein rZIP R02750

POSITION	↓ITC (AD/C)	MFC (AD/C)	OP (AD/C)	GENE GBACCESSION#
3_b01_7	(>∞x↓) 0+0 /1276+1799+546	↑ 1830+2507 /765	↑ 1295+1475+605 /481	(unknown) W87710

[TABLE 3B: "↑ ITC" = Up-regulated Genes in ITC region of AD brain]

POSITION	↑ ITC (AD/C)	MFC (AD/C)	OP (AD/C)	GENE GBACCESSION#
1_h23_5	>64x↑ 1058+577 /0+9+0	637+1156 /1178	↑ 13+548+942 /0	**Homologous to *A. japonica* androgen receptor alpha** AB023960
5_a04_8	>3x↑ 786+1951 /0+0+258	1731+1609 /1720	↑ 2371+2297+1385 /468	**VDAC1** Outer Mitochondrial membrane protein (aka Porin) AA025089
2_d06_4	(>∞x↑) 1123+2569 /0+0+0	↓ 0+176 /872	↓ 0+0+25 /2447	**Human Retrovirus mRNA** for solo LTR #2 X12724
3_d24_2	(>∞x↑) 4290+2694 /0+0+0	0+2780 /229	↓ 0+0+1774 /3091	**Putative Receptor Protein** T90668
6_c23_5	(>∞x↑) 7776+6411 /0+0+0	↑ 5329+5175 /1949	↑ 5517+3542+3887 /102	**Lysosomal Acid Phosphatase** N23945
6_k14_6	>66x↑ 1262+6148 /0+18+0	1108+6349 /2090	↑ 3210+7007+725 /0	**Forkhead protein FREAC-2** N98485
"4_h09_3" (*)	>2.5x↑ 1637+3777 /0+189+630	343+2931 /1961	1652+2294+1530 /1768	**AP-2 gene** R71483
1_p13_2	(>∞x↑) 2175+1801 /0+0+0	0+5521 /2936	3845+209+2010 /1537	**Beta-Adaptin** H11628
1_p13_4	(>∞x↑) 1516+2940 /0+0+0	0+4468 /3129	2863+685+2113 /1401	**Kir3.2** R87988
5_l13_6	>74x↑ 1401+1459 /19+0+0	709+1752 /2159	0+0+1532 /635	**Uncharacterized protein KIAA0550** AB011122

POSITION	↑ITC (AD/C)	MFC (AD/C)	OP (AD/C)	GENE GBACCESSION#
6_k14_7	>21x↑ 842+8863 /0+0+39	↑ 2616+9575 /1066	↑ 5708+9743+764 /0	**Hermansky-Pudlak syndrome protein** U65676
2_d20_8	>6x↑ 850+1298 /0+92+140	0+651 /433	↓ 0+0+109 /437	**Parathymosin** AA125890
"4_e04_8" (*)	>5x↑ 1319+1615 /0+177+259	↑ 1398+2121 /974	↑ 1014+1101+930 /715	**FIBULIN-1,** (isoformic precursor) AA142940
4_j12_7	(>∞x↑) 2050+2430 /0+0+0	1624+2263 /2220	2519+1013+146 3 /1161	**rasGAP-associated protein p190** AA010440 (80/97)
1_p13_1	(>∞x↑) 4319+3066 /0+0+0	278÷5639 /6255	4522+2803+406 3/2008	Homologous to Zn finger Transcription Factor R38905
6_c23_3	(>∞x↑) 2479+3474 /0+0+0	1025+2937 /939	↑ 3393+1833+172 0/0	Homologous to Acetylcholinesterease H03710
1_n17_7	(>∞x↑) 1891+5588 /0+0+0	↑ 3728+5103 /2082	↑ 4212+1825+0 /0	Homologous to Putative Tyrosine Protein Kinase W02842
4_o12_7	(>∞x↑) 2314+3290 /0+0+0	↓ 1497+1037 /3478	↑ 2869+354+0 /0	**Uncharacterized protein** AA011654
4_o12_1	(>∞x↑) 4230+1442 /0+0+0	0+1111 /1009	↑ 2405 /0+0+0	**Uncharacterized protein** R46079
4_d11_6	(>∞x↑) 1667+2747 /0+0+0	↑ 2205+2911 /1398	↑ 2610+3137+160 5 /63	**Homologous to Glutamine Synthetase** N73996
1_p13_3	(>∞x↑) 3072+3447 /0+0+0	0+5239 /4357	↑ 4244+3548+391 8/2115	**Homologous to Fimbrial-associated proteins or Collagen Alpha LPHA 2(IX) Chain** R10152

POSITION	↑ ITC (AD/C)	MFC (AD/C)	OP (AD/C)	GENE GBACCESSION#
4_c02_1	>40↑ 986+1226 /0+0+25	↓ 0+640 /3857	0+3151+0 /0	Fln mRNA R45593

[Table 3C: "↓ MFC" = Down-regulated Genes in MFC region of AD brain]

POSITION	ITC (AD/C)	↓MFC (AD/C)	OP (AD/C)	GENE GB_ACCESSION#
5_d01_8	346+1859 /4447+382+968	>10x↓ 0+171 /1760	↑ 4330+2092+1878 /774	**Peroxisome Assembly Factor-1** AA194896
5_k10_5	3142+1441 /3437+3218+3743	>11x↓ 0+481 /5073	2913+6668+0 /767	Polypyrimidine Track-binding protein **N24229**
3_g17_4	1333+0 /538+0+0	↓ 0+0 /5873	1103+0+0 /238	**Transcription Factor ITF-2** H28074
5_a08_8	868+1489 /477+2190+167	↓ 0+0 /1475	↑ 1966+1350+1090/ 263	**CGMP-gated Cation channel protein** W84526
5_b02_2	5364+4419 /1876+1415+6304	↓ 0+0 /7354	↑ 3437+5163+3757 /0	Cyt P450 IIIA5 T95816
5_a13_5	128655+24581 /72612+88028+ 135994	>4.5x↓ 51898+689 05 / 311593	↑ 243855+181784+2 41096 /84669	Aldehyde DH6 **N32240**
1_j14_8	648+377 /718+53+554	>3.7x↓ 0 + 531 / 2005	↑ 904+1340+1354 /157	**6-Pyruvoyl Tetrahydrobiopteri n,** AA045810
5_h13_3	2574+2448 /1929+808+1670	>2.3x↓ 1835 +517 / 4221	2973+3195+701 /1641	**Mitochondrial short-chain enoyl-CoA hydratase** H04530
5_a09_3	588+1746 /490+3132+1975	>2x↓ 783+496 / 1547	3232+2519+1665 /2150	**Biliverdin-IX alpha reductase** R78220
6_k04_4	2501+1768 /2055+1412+2294	>10x↓ 910+2742 / 28182	775+1993+1406 /2323	**Glycophorin E** H60621

[Table 3D: "↑ MFC" = Up-regulated Genes in MFC region of AD brain]

POSITION	ITC (AD/C)	↑MFC (AD/C)	OP (AD/C)	GENE GB_Accession#
6_k01_1	3037+2412 /1973+0+1814	↑ 915+4815 /0	618+2399+3120 /4583	**Disulfide Isomerase-related Protein** R59974
6_f08_6	1046+2370 /1940+1031+1212	↑ 951+4405 /0	2494+1897+778 /1582	**Membrane-associated protein HEM-1** W33170
5_d22_5	2332+2369 /1165+2833+2679	↑ 2123+3174 /0	2647+2911+462 /528	Low protein homology to glutathione S-transferase N20843
2_o08_7	>1.5x↑ 2286+3099 /636+644+1531	↑ 2487+5749 /0	3935+3266+1479 /3392	**Homologous to Putative Zinc Finger Protein** AA058580
2_a19_1	848+3368 /747+907+525	>4.7x↑ 576+2682 / 122	244+3462+790 /1330	**Complement component C4A** R37128
4_o09_8	366+1337 /347+461+925	>8.7x↑ 656+878 / 75	1108+415+655 /604	Homologous to EF Hand Domain (EFHD-like Protein) AA132078
3_b01_4	(∞↓) 0+0 /1109+1787+240	>75x↑ 1992+1649 / 22	928+1178+590 /755	**Ig gamma & kappa chain V-III** R83214
2_f16_8	848+136 /358+454+1205	>13x↑ 1969+1142 / 88	5235+1462+355 /1857	**Calcium-binding protein S100P** AA136673
2_m03_2	1435+597 /648+1443+5006	>11x↑ 1389+1416 / 125	2170+1804+315 /3419	**Lysosomal acid lipase, choles-teryl ester hydrolase (LIPA)** H15248

[TABLE 3E: "↓ OP" = DOWN-REGULATED GENES IN OP REGION OF AD BRAIN]

POSITION	ITC (AD/C)	MFC (AD/C)	↓OP (AD/C)	GENE GB_ACCESSION#
5_f20_5	1488+1088 /1430+1179+1591	2328+1678 /1111	↓ 1187+1564+105 /11326	Homologous to Insulin-like Growth Factor IA N25355
1_d20_8	↑ 1484+3472 /382+448+362	↑ 1179+2504 /0	↓ 0+701+0 /2388	**S19 ribosomal protein** AA046433
2_c10_1	7740+32 /0+0+2346	↑ 308+519 /0	>3.7x↓ 689+0+0 /2527	Protein Phosphatase PP2A_ R37078
4_p17_6	663+544 /628+164+2560	↑ 1502+1534 /777	>5.7x↓ 378+1321+339 /7547	**L11 ribosomal protein** N68904
4_a16_3	↓ 9989+1786 /103380+106691 +60720	13007+22 /12100	>4.6x↓ 4429+28+18269 /83633	Bone/cartilage Proteoglycan I **R63813**
	↑		>4x↓	Parathymosin **AA125890**
3_i01_8	1291+79 /1434+1039+2038	1551+60 /701	↓ 0+0+368 /2183	Porcollagen _1 (IV) **AA150402**
2_p02_7	4177+0 /0+0+0	236+21366 /289	>6.1x↓ 0+154+0 /933	CDC25 M-Phase Inducer Phosphatase 3 **W95001**
1_d20_7	↑ 1588+3005 /354+384+0	0+706 /0	↓ 0+577+0 /4167	HLA Class II Histo-compatibility Ag, DP(W2) **W20380**

[TABLE 3F: "↑ OP" = UP-REGULATED GENES IN OP REGION OF AD BRAIN]

POSITION	**ITC (AD/C)**	**MFC (AD/C)**	**↑OP (AD/C)**	Gene **GB_Accession#**
5_b02_2	5364+4419 /1876+1415+6304	↓ 0+0 /7354	↑ 3437+5163+3757 /0	**Cyt P450 IIIA5** T95816
6_b12_3	4544+492 /1910+0+877	1742+5040 /740	↑ 6009+3490+2786 0	B94 Protein R54702
5_108_4	1317+2118 /283+1138+0	1172+1303 /2525	↑ 1134+9266+1739 /0	Protein Kinase C Theta **H60824**
5_n11_3	↑ 547+4067 /0+0+0	6440+1629 /3085	↑ 1647+6261+3257 /0	Pregnancy-specific beta-1 Glycoprotein **H01531**
5_m21-4	>1.8x↑ 4980+3041 /515+466+1663	10569+2955 /2166	↑ 5508+3130+6007 /0	PLK-1 **R99810**
5_b23_6	**C.** **D. 1756+1663** **E. /412+1268 +2390**	468÷1776 /579	↑ 30517+957+877 /0	Prostacyclin-stimulating factor **N80187**
1_c03_4	4024+130961 /0+76974+19539	↑ 167032 +192037 /38185	↑ 50361+135914 +57076 /0	Glycogen Phosphorylase **(liver form)** **R74161**

(*) Indicates array positions that apparently did not contain the manufacturer-indicated gene sequence. Accordingly, the identities of the proteins (*i.e.*, gene products) described in these instances is based on the nucleotide sequences of these genes, and not on the manufacturer's description.

EXAMPLE 7

QUANTITATIVE REAL-TIME PCR ANALYSIS OF AD DIFFERENTIALLY EXPRESSED GENES

Quantitative real-time PCR (Q-RTPCR) was used to measure the expression of several of the AD differentially expressed genes described in the preceding Example. The Q-RTPCR techniques, as they were applied to contactin sequences, are described in detail in Example 2. In the present Example the same basic techniques were performed essentially as in the preceding Examples, with the following exceptions, as detailed below.

Each pair (one forward, one reverse) of oligonucleotide primers had sequences derived not from contactin but from each AD differentially expressed gene for the Q-RTPCR experiments. Oligonucleotide probes having sequences specifically hybridizable to each of the AD differentially expressed genes were also prepared (see "Table B"). Specific nucleic acids were detected and measured by Q-RTPCR and the ABI PRISM 7700 Sequence Detection System using the primers and SYBR® Green PCR reagents.

In order to correct for sample-to-sample variation, an internal RNA normalizer was used in Q-RTPCR. The RNA normalizer was selected from an endogenous RNA species such as an mRNA encoding a constitutively-expressed protein like actin or glyceraldehyde-3-phosphate dehydrogenase (GAPDH), or a ribosomal RNA such as 18S or 28S rRNA. RNA molecules produced *in vitro* may also be used as normalizers. Results of Q-RTPCR analyses are thus often expressed as relative amounts. The relative amount of the AD differentially expressed RNA was determined as compared to the normalizer essentially according to protocols and formulae described in the manufacturer's (PE Corp., PE Applied Biosystems Division, Foster City, CA) instructions entitled "User Bulletin #2 (version of December 11, 1997), ABI PRISM 7700 Sequence Detection System, section entitled "Comparative C_T Method, pages 11-15; some of these formulae are provided in Example 2 above.

Human brain samples were promptly obtained from various regions of the brain (*e.g.*, inferior temporal cortex (ITC), medial frontal cortex (MFC), occipital

pole (OP), temporal parietal cortex and cerebellum). RNA was isolated from the biological samples essentially according to the methods described in Example 3. In brief, total RNA was isolated from the biological samples using the TRIzol® Reagent supplied by Life Technologies™ essentially following the manufacturer's instructions (one version of the TRIzol® Reagent is described in U.S. Patent No. 5,346,994). Briefly, tissue samples were homogenized in TRIzol® Reagent (2 mL of TRIzol® Reagent per 50 mg to 100 mg of tissue). The homogenized samples were incubated at about 15°C to 30°C for about five minutes. Chloroform (0.2 ml per ml of TRIzol® Reagent used in the initial homogenization) was added and the mixture shaken vigorously for fifteen seconds, incubated at about 15°C to 30°C for about two or three minutes, and centrifuged at about 12,000 x g for fifteen minutes at about 2°C to 8°C. The aqueous phase was transferred to a fresh container and the RNA therein was precipitated by mixing with 0.5 ml of isopropyl alcohol per 1 ml of TRIzol® Reagent used in the initial homogenization. The mixture was incubated at about 15°C to 30°C for about 15 minutes and centrifuged at about 12,000 x g for 15 minutes at about 2°C to 8°C. The supernatant was removed, and the resulting pellet was washed once with 75% ethanol and recollected by centrifugation at about 7,500 x g for five minutes at about 2°C to 8°C. The resulting pellet was collected, dried and resuspended in buffer.

Data showing the expression of ten AD differentially expressed genes, as determined by Q-RTPCR, are shown in Table 5. The data in Table 5 are expressed as a ratio of the level of expression in AD samples as compared to the level of expression in control (C) brains. A ratio greater than 1.0 indicates that a genetic sequence that is up-regulated in AD brains relative to control brains, whereas a ratio of less than 1.0 indicates that a genetic sequence that is down-regulated in AD brains relative to control brains.

EXAMPLE 8

CHARACTERISTICS AND FUNCTIONS OF GENES THAT ARE DIFFERENTIALLY EXPRESSED IN NEURODEGENERATIVE DISEASES.

Without wishing to be bound by any theory, the above-described genes (and proteins encoded thereby) that are differentially expressed in neurodegenerative disease, have characteristics and functions that have been reported in the scientific literature. Summaries of research to date for each of the differentially expressed genes and the products thereof, where known, are provided below.

Contactin

Human contactin protein, which is up-regulated in MS brains as described in the preceding Examples, and its homologs from mouse (F3 protein) and chicken (F11 protein), are cell surface adhesion proteins that are involved in cell attachment to substrate. Contactin comprises an Ig-like domain and multiple fibronectin III-like domains (Brummendorf et al., *J. Neurochemistry* 61:1207-1219 (1993)). Unlike many cell adhesion molecules, contactin is not a transmembrane protein, but instead is anchored to cell surfaces via linkage to glycosylphosphatidylinositol (GPI) in the plasma membrane outer leaflet (*Id.*). In human tissues, relatively high levels of a major contactin mRNA (6.5 kb) are expressed in adult brain along with three minor transcripts (9.7 kb, 4.4 kb and 3.4 kb), whereas low levels of expression of multiple forms of contactin mRNA are found in the adult lung, pancreas, kidney and skeletal muscles (6.8 kb and 6.0 kb) (Reid et al., *Molecular Brain Research* 21:1-8 (1994)). High levels of expression of the multiple forms of contactin mRNA are found in neuroblastoma and retinoblastoma cell lines (6.8 kb, 6.0 kb and 4.2 kb) (Id.). The expression of contactin in developing neural tissue is complex, transient, and temporally regulated. Contactin is believed to have a role in neurite outgrowth, perhaps by binding to the cell recognition molecule Ng-CAM and/or by interacting with the extracellular matrix glycoprotein restrictin (Faivre-Sarrailh et al., *J. Neurosci.* 12:257-267 (1992), Brummendorf et al., *Neuron* 10:711-7272 (1993)).

Adult neural stem cells can give rise to hematopoietic cells, including cells of the myeloid and lymphoid lineages (Bjornson et al., *Science* 283:534-537 (1999)); thus, contactin mRNA, contactin DNA or contactin protein may be detected in blood.

COX7c

COX7c, which is down-regulated in AD brains as described in the preceding Examples, corresponds to the VIIc subunit of Complex IV (cytochrome c oxidase) of the ETC chain. In mammals, COX7c is a mitochondrial protein that is encoded by nuclear genome, not the mitochondrial genome. A number of previously-described assays for activities and properties of COX7c, and/or subunit IV of the ETC, may serve as a useful screening assays for identifying chemical and biological treatments for AD and other neurodegenerative disorders.

FREAC-2

Forkhead Related Activator-2 (FREAC-2), which is up-regulated in AD brains as described in the preceding Examples, was initially cloned from a human cDNA library by Pierrou et al. (*EMBO J.* 13:5002, 1994). FREAC-2 is a member of the family of "winged helix" or "forkhead" transcription factors (for a review, see Kaufmann et al., *Mechanisms of Development* 57:3-20, 1996). FREAC-2 binds to DNA as a monomer and plays regulatory roles in embryogenesis, tumorigenesis, and the maintenance of differentiated cells. Published reports suggest that expression of FREAC-2 is limited to lung and placenta; accordingly, the elevated expression of FREAC-2 in AD brains may be an abnormal process that plays a role in the development and/or maintenance of AD. There are various activities of FREAC-2, including without limitation its binding to DNA (Hellqvist et al., J. Biol. Chem. 271:4482-4490, 1996).

APCL

The APCL ("Adenomatous Polypopsis Coli Like") gene, which is up-regulated in AD brains as described in the preceding Examples, encodes a protein of 2023 amino acid residues that is expressed specifically in the brain. APCL has a variety

of *in vitro* and *in vivo* activities and functions, each of which has been examined to some degree, that might relate to its role in AD and other neurodegenerative disorders.

For example, APCL binds to beta-catenin *in vitro*, and overexpression of APCL in SW480 cells results in a reduction of the intracellular beta-catenin pool (Nakagawa et al., *Cancer Research* 58:5176-5181, 1988). Up-regulation of APCL, as seen in AD, is thus expected to reduce intracellular levels of beta-catenin. Beta-catenin levels are reduced, and/or beta-catenin proteins are inappropriately targeted intracellularly, in the brains of AD patients who also have mutations in presenilin-1, a protein that complexes with and stabilizes beta-catenin (Zhang et al., *Nature* 395:698-702, 1998; Nishimura et al., *Nature Medicine* 5:164-169, 1999). Moreover, reduced beta-catenin signalling increases neuronal vulnerability to apoptosis induced by amyloid-beta protein and oxidative stress (Zhang et al., Nature 395:698-702, 1998).

Members of the Tcf family of transcription factors, in association with beta-catenin, mediate the transactivation of downstream genes involved in neural development (Cho et al., *Mechanisms of Development* 77:9-18, 1998). Thus, according to non-limiting theory, beta-catenins may be involved in the development or propagation of AD and other neurodegenerative disorders.

LAP

Lysosomal acid phosphatase (LAP) is a tartrate-sensitive enzyme with ubiquitous expression that is up-regulated in AD brains. Neither the physiological substrates nor the functional significance of LAP is known. Mice with a deficiency of LAP generated by targeted disruption of the LAP gene are fertile and develop normally, and microscopic examination of various peripheral organs reveals progredient lysosomal storage in podocytes and tubular epithelial cells of the kidney, with regionally different ultrastructural appearance of the stored material. Within the central nervous system of such mice, lysosomal storage was detected to a regionally different extent in microglia, ependymal cells, and astroglia concomitant with the development of a progressive astrogliosis and microglial activation. Although behavioral and neuromotor analyses were unable to distinguish between control and deficient mice,

approximately 7% of the deficient animals developed generalized seizures. (Saftig et al., *J. Biol. Chem.* 272:18628-18635, 1997).

PAF

Peroxisome Assembly Factor-1 (PAF), which is down-regulated in AD, is required for the normal assembly of peroxisomes in the liver and other organs. Defects in peroxisome assembly have many deleterious effects, including the widespread distribution of iron in all organs that is seen in Zellweger Syndrome and other disorders. Zellweger Syndrome is typically fatal by the age of 6 months, but some initial attempts at evaluating the potential for gene therapy for peroxisome assembly defects have been promising (Young, S.P. and Aisen P.: *The Liver and Iron, in The Liver Biology and Pathobiology* (I.M. Arias, J.L. Boyer, N. Fausto, W.B. Jakoby & D. Schachter, Eds.) pp. 609, Raven Press, New York, 1994.).

6PTS-1

The 6-pyruvoyl-tetrahydropterin synthase (6PTS-1, a.k.a. PTPS), down-regulated in AD, is the second enzyme in the biosynthetic pathway from GTP to tetrahydrobiopterin (BH4). In turn, BH4 is an essential cofactor of NO synthases and aromatic amino acid hydroxylases, the latter being responsible for, inter alia, monoamine neurotransmitter biosynthesis (Turri et al., *Biol. Chem.* 379:1441-1447, 1998). Thus, down-regulation of 6PTS-1 may lead to decreased synthesis of certain neurotransmitters in AD and other neurodegenerative disorders

VDAC-1

VDAC-1, down-regulated in AD brains, is one of several voltage-dependent anion channels (VDACs, also known as mitochondrial porins). VDACs are small pore-forming proteins of the mitochondrial outer membrane found in all eukaryotes, and are the binding sites for several cytosolic enzymes, including the isoforms of hexokinase and glycerol kinase. VDACs have also been shown to conduct ATP when in the open state, allowing bound kinases preferential access to mitochondrial ATP and providing a possible mechanism for the regulation of adenine

nucleotide flux. (for reviews, see Sampson et al., *J. Biol. Chem.* 272:18966-18973, 1997; Mannella, *J. Bioenerg. Biomembr.* 29:6525-6531,1997).

Several VDAC isoforms are found in mammals (Blachly-Dyson et al. *Genomics* 20:162-167, 1994). In vitro, VDACs conduct a variety of small metabolites and in vivo they serve as a binding site for several cytosolic kinases involved in intermediary metabolism, yet the specific physiologic role of each isoform is unknown. Mouse embryonic stem cells lacking each isoform are viable but exhibit a 30% reduction in oxygen consumption. VDAC-1 and VDAC-2 deficient cells exhibit reduced *cytochrome c oxidase activity, whereas VDAC-3 deficient cells have normal activity (Wu et al., Biochim. Biophys. Acta* 1452:168-178, 1999).

It has been suggested that the mitochondrial permeability transition (MPT) pore may comprise VDAC molecules (Szabó et al., *FEBS Lett.* 330:206-10, 1993). In support of this proposal, in vitro experiments have demonstrated that the pro-apoptotic proteins Bax and Bak accelerate the opening of VDAC, whereas the anti-apoptotic protein Bcl-x(L) closes VDAC by binding to it directly. Bax and Bak allow cytochrome c to pass through VDAC out of liposomes, but such passage is prevented by Bcl-x(L). These results indicate that the Bcl-2 family of proteins bind to the VDAC in order to regulate the mitochondrial membrane potential and the release of cytochrome c during apoptosis (Shimizu et al., *Nature* 399:483-487, 1999).

Brain-Expressed Novel EFHD-Like Protein

A number of EF-hand (EFHD) proteins have been identified, so many so that over 40 distinct subfamilies are recognized (Kawasaki et al., *Biometals* 11:277-295, 1998). The EF-hand motif consists of two alpha helices, "E" and "F", joined by a Ca(2+)-binding loop. EF-hands have been identified in numerous Ca(2+)-binding proteins by similarity of amino acid sequence and confirmed in some crystal structures. Functional EF-hands seem to tend to occur in pairs. To date, the EF-hand homolog family contains more than 160 different Ca(2+)-modulated proteins which have a broad range of functions. Among them, are the calmodulin, the troponin C, the myosin

regulatory light chain, the parvalbumin, the S-100 proteins and the calbindins 9- and 28 kDa.

The most striking features of members of the EF-hand family are the ability to bind calcium (Ca2+) with dissociation constants in the micromolar range and the ability to modulate the activity of a number of enzymes. The activity of EHFD proteins is generally thought to be modulated by stimulus-induced increases in cytosolic free Ca2+ (Weinman, *J. Biol. Buccale* 1:90-98,1991). Except for troponin-C, all subfamilies and unique EF-hand homologs represented in vertebrates can be found in the CNS (Persechini et al., *Trends Neurosci.* 11:462-467. 1989). The Brain-Expressed Novel EFHD-Like Protein described herein is up-regulated in AD as described in the preceding Examples. Assays of various activities and properties of the Brain-Expressed Novel EFHD-Like Protein, including but not limited to intracellular calcium assays As a non-limiting example, one set intracellular calcium assays that may be used in this application are described, for example, in copending U.S. patent application Serial No. 60/176,384, hereby incorporated by reference.

EXAMPLE 9

QUANTITATIVE REAL-TIME PCR ANALYSIS OF EXPRESSION OF AD DIFFERENTIALLY EXPRESSED GENES IN OTHER NEURODEGENERATIVE DISEASES.

The present invention can be used to identify genes that are differentially expressed in a consistent manner in a variety of neurodegenerative diseases ("shared genes") as well as those that are differentially expressed in one particular neurodegenerative disease but not in others ("disease-specific genes"). The expression patterns in neurodegenerative diseases other than AD of several AD up- and down-regulated genes from the preceding Example were further examined as follows.

Quantitative real-time PCR (Q-RTPCR) was used to measure the expression of these genes in samples from other diseases. Human brain samples were obtained from the various regions (for *e.g.*. inferior temporal cortex (ITC), medial

frontal cortex (MFC), occipital pole (OP), temporal parietal cortex and cerebellum) post mortem from decedents as soon as possible after death. Samples were obtained from a patient diagnosed with Alzheimer's Disease (AD), Vascular Dementia (VaD), Diffused Lewy Body Disease (DLB), Parkinsons' Disease (PD) and a control (C) patient. The biological samples were frozen and stored at -80°C, and shipped on dry ice.

Q-RTPCR was performed on the brain samples from decedents having one or more neurodegenerative disorders using primers and probes specific for VDAC-1, FREAC-2, COX7c, and APCL. The results from these experiments were grouped according to evaluations of the stage and degree of AD in each decedent, i.e., "sAD" denotes severe AD, "mAD" denotes mild AD, and "No AD" indicates decedents not having characteristics of AD. It should be noted, however, that decedents in each of these classes of AD may nonetheless have one or more other neurological disorders such as, e.g., PD.

The results of these experiments regarding VDAC-1, FREAC-2, COX7c, and APCL are shown in Tables 6, 7, 8 and 9, respectively. The Q-RTPCR primers and probes were used in the reactions as follows. Each probe was designed to be complementary to a portion of an mRNA of interest (*i.e.*, having an antisense sequence as compared to the sense strand present in mRNA). The portion to which the probe hybridized was located between the two primers used for amplification, that is, within the amplification products. The target mRNA was labeled with a fluorescent dye (a "quencher") that served to quench the signal from a second fluorescent dye attached to the oligonucleotide probe. TAMRA was typically used as the quencher, and a variety of dyes can be used for probe labeling (*e.g.*, 6-FAM, TET, JOE and the like).

Q-RTPCR was performed according to instructions provided by the instrument manufacturer (see http://www.pebiodocs.com/pebiodocs/04303859.pdf, which is incorporated by reference, in particular pages 11-15 therein.) Before the polymerase chain reactions were initiated, the probes specifically hybridized to complementary sequence in the mRNA of interest. The fluorescent signal from the probe was suppressed due to the close proximity of the probe's dye to the quencher dye present in the mRNA. Thus, at time = 0, little or no fluorescence from the dye linked

to the probe was detected. During PCR, however, the labeled probes were separated from the mRNA to which they hybridize due to the helicase and/or exonuclease activity of the polymerase. In either event, the dye initially present in the probe became physically separated from the quencher molecules as the PCR continued and, as a consequence, the fluorescent signal corresponding to displaced probe molecules increased over time. This signal was monitored over time and reflects the amount of the mRNA of interest present in the sample (*i.e.*, a greater amount of mRNA molecules required more PCR cycles, and/or different concentrations of primers and probes, to separate the quencher dye molecules from the reporter dye molecules. These parameters and measurements were used to determine the amount of an mRNA of interest in a sample.

Table 6

Relative VDAC1 mRNA Levels [ΔCt value (VDAC1-Actin)] in Human AD Brain and Other Neurological Diseases

Pathologist's Diagnosis	Patient	Δ Ct TPC (Temporal Parietal Cortex)		Δ Ct Cere (Cere-bellum)		ΔΔ Ct = Δ Ct TPC - Δ Ct Cere	$2^{-\Delta\Delta Ct}$ = Fold Change	Fold Change In Sub Patient Groups	
	ID#	Mean	SD	Mean	SD		Per Patient	Mean	SD
A98-01/sAD (Braak stage VI)	sAD #1	4.205	0.332	3.718	0.143	0.487	0.714		
A98-07/AD (Braak stage VI and DLB)	sAD #2	3.658	0.313	3.324	0.152	0.333	0.794		
A97-7/sAD (Braak stage VI)	sAD #3	4.167	0.094	4.518	0.139	-0.352	1.276	0.798	0.281
A97-9/sAD (Braak stage VI and DLB)	sAD #4	5.105	0.127	4.25	0.392	0.855	0.553		
A96-8/sAD (Braak stage VI)	sAD #5	4.393	0.262	3.779	0.240	0.613	0.654		
Temporal Parietal Cortex: Mean | SD		4.305	0.523						
Cerebellum: Mean | SD				3.918	0.470				
A98-18/AD (V)	mAD #1	3.484	0.314	3.216	0.194	0.268	0.830		
A96-25/AD (IV)	mAD #2	3.992	0.055	4.278	0.246	-0.286	1.219		
A97-05/mAD (V)	mAD #3	5.067	0.221	3.466	0.158	1.601	0.330	0.728	0.351
A97-10/sAD (V and DLB)	mAD #4	4.056	0.360	3.085	0.503	0.971	0.510		
A96-23/AD III and VaD and DLB	mAD #5	4.069	0.190	2.943	0.099	1.127	0.458		
A98-04/limbic AD III	mAD #6	3.828	0.182	3.860	0.134	-0.032	1.022		
Temporal Parietal Cortex: Mean | SD		4.083	0.529						
Cerebellum: Mean | SD				3.475	0.509				

Pathologist's Diagnosis	Patient ID#	Δ Ct TPC (Temporal Parietal Cortex) Mean	SD	Δ Ct Cere (Cere-bellum) Mean	SD	ΔΔ Ct = Δ Ct TPC - Δ Ct Cere	$2^{-\Delta\Delta Ct}$ = Fold Change Per Patient	Fold Change In Sub Patient Groups Mean	SD
A96-11/VaD (no AD change)	No AD #1	4.417	0.157	3.746	0.149	0.671	0.628		
A97-11/mixed (corticobasal degeneration and PD)	No AD #2	3.742	0.033	3.757	0.289	-0.015	1.010		
A97-18/VaD (no AD changes)	No AD #3	3.561	0.097	3.656	0.262	-0.095	1.068		
A97-19/VaD (no AD changes)	No AD #4	4.023	0.039	3.852	0.205	0.171	0.888	0.854	0.294
A96-6/DLB (no AD)	No AD #5	5.448	0.276	3.673	0.220	1.775	0.292		
A96-12/DLB (no AD)	No AD #6	4.158	0.185	4.129	0.128	0.028	0.981		
A95-48/DLB (no AD)	No AD #7	3.955	0.127	4.107	0.098	-0.152	1.111		
Temporal Parietal Cortex: Mean | SD		4.186	0.621						
Cerebellum: Mean | SD				3.845	0.197				

Table7

Relative FREAC-2 mRNA Levels in Human Brain ["delta"Ct Value (FREAC-2 – Actin)] in Human Brain Samples from AD and Other Neurological Diseases

	Pathologist's Diagnosis	Patient ID#	DCt TPC (Temporal Parietal Cortex) Mean	SD	DCt Cere (Cerebellum) Mean	SD	DDCt = D Ct TPC - D Ct Cere.	2^{-Ct} = Fold Change per Patient	Fold Change In Sub Patient groups Mean	SD
1	**A98-19,11 (Control)**	Control	11.375	0.234	12.130	0.118	-0.755	1.688	N/A	N/A
2	**A98-01/sAD (Braak stage VI)**	sAD #1	10.565	0.233	11.522	0.204	-0.957	1.941		
3	**A98-07/AD (Braak stage VI and DLB)**	sAD #2	10.302	0.273	11.468	0.274	-1.166	2.244		
4	**A97-7/sAD (Braak stage VI)**	sAD #3	9.663	0.229	11.088	0.125	-1.425	2.685	3.484	1.657
5	**A97-9/sAD (Braak stage VI and DLB)**	sAD #4	9.011	0.177	11.395	0.141	-2.384	5.220		
6	**A96-8/sAD (Braak stage VI)**	sAD #5	9.720	0.256	12.134	0.251	-2.414	5.329		

	Pathologist's Diagnosis	Patient ID#	DCt TPC (Temporal Parietal Cortex)		DCt Cere (Cerebellum)		DDCt = D Ct TPC - D Ct Cere.	2^{-Ct} = Fold Change per Patient	Fold Change In Sub Patient groups	
			Mean	SD	Mean	SD			Mean	SD
7	Temporal Parietal Cortex: Mean | SD		9.852	0.607						
8	Cerebellum: Mean | SD				11.521	0.381				
9	A98-18/AD (V)	mAD #1	11.617	0.154	12.293	0.236	-0.676	1.598		
10	A96-25/AD (IV)	mAD #2	12.505	0.421	13.586	0.667	-1.081	2.116		
11	A97-05/mAD (V)	mAD #3	11.041	0.122	10.907	0.301	0.134	0.911		
12	A97-10/sAD (V and DLB)	mAD #4	11.467	0.279	12.310	0.339	-0.843	1.794	1.825	0.934
13	A96-23/AD III and VaD and DLB	mAD #5	9.220	0.320	11.023	0.209	-1.803	3.489		
14	A98-04/limbic AD III	mAD #6	11.395	0.141	11.452	0.026	-0.057	1.040		
15	Temporal Parietal Cortex: Mean | SD		11.208	1.089						
16	Cerebellum: Mean | SD				11.928	1.012				
17	A6-11/VaD (no AD change)	No AD #1	12.000	0.035	11.852	0.439	0.148	0.903		
18	A97-11/mixed (corticobasal degeneration and PD)	No AD #2	11.208	0.209	13.000	0.409	-1.792	3.463		
19	A97-18/VaD (no AD changes)	No AD #3	11.501	0.205	11.757	0.267	-0.256	1.194		
20	A97-19/VaD (no AD changes)	No AD #4	11.634	0.208	12.714	0.145	-1.080	2.114	1.637	0.915
21	A96-6/DLB (no AD)	No AD #5	11.222	0.248	11.364	0.237	-0.142	1.103		
22	A96-12/DLB (no AD)	No AD #6	12.319	0.189	13.086	0.237	-0.767	1.702		
23	A95-48/DLB (no AD)	No AD #7	11.190	0.105	11.158	0.452	0.032	0.978		
24	Temporal Parietal Cortex: Mean | SD		11.582	0.438						
25	Cerebellum: Mean | SD				12.133	0.792				

Table 8

Relative COX7c mRNA Levels in Human Brain ["delta"Ct Value (COX7c – Actin)] in Human Brain Samples from AD and Other Neurological Diseases

Diagnosis	ID#	ΔCt TPC Mean	ΔCt TPC SD	ΔCt Cere ΔCt Ave	ΔCt Cere SD	ΔΔCt = ΔCt Cere	2^-ΔΔCt = Per Patient	Fold Change Mean	Fold Change SD
A98-19,11 (Control)	Control	4.503	0.100	4.439	0.126	0.063	0.957	N/A	N/A
A98-01/sAD (Braak stage VI)	sAD #1	4.626	0.387	4.188	0.278	0.438	0.738		
A98-07/AD (Braak stage VI and DLB)	sAD #2	4.905	0.264	4.355	0.194	0.550	0.683		
A97-7/sAD (Braak stage VI)	sAD #3	4.639	0.283	4.605	0.286	0.034	0.977	0.781	0.129
A97-9/sAD (Braak stage VI and DLB)	sAD #4	4.535	0.197	4.286	0.219	0.249	0.841		
A96-8/sAD (Braak stage VI)	sAD #5	4.886	0.196	4.301	0.091	0.585	0.667		
Temporal Parietal Cortex: Mean | SD		4.718	0.167						
Cerebellum: Mean | SD				4.347	0.156				
A98-18/AD (V)	mAD #1	4.498	0.111	4.195	0.291	0.302	0.811		
A96-25/AD (IV)	mAD #2	4.953	0.171	4.994	0.215	-0.041	1.029		
A97-05/mAD (V)	mAD #3	5.252	0.133	4.349	0.244	0.903	0.535	0.885	0.403
A97-10/sAD (V and DLB)	mAD #4	4.396	0.251	3.683	0.272	0.713	0.610		
A96-23/AD III and VaD and DLB	mAD #5	3.146	0.173	3.848	0.131	-0.702	1.627		
A98-04/limbic AD III	mAD #6	4.767	0.116	4.243	0.231	0.524	0.696		
Temporal Parietal Cortex: Mean | SD		4.502	0.733						
Cerebellum: Mean | SD				4.219	0.457				
A96-11/VaD (no AD change)	No AD #1	5.181	0.195	3.949	0.140	1.232	0.426		
A97-11/mixed (corticobasal degeneration	No AD #2	4.704	0.063	4.295	0.108	0.409	0.753		

Diagnosis	ID#	ΔCt TPC Mean	SD	ΔCt Cere ΔCt Ave	SD	ΔΔCt = ΔCt Cere	2^-ΔΔCt = Per Patient	Fold Change Mean	SD
& PD)									
A97-18/VaD (no AD changes)	No AD #3	4.828	0.102	4.736	0.096	0.092	0.938	0.692	0.189
A97-19/VaD (no AD changes)	No AD #4	5.195	0.070	4.713	0.121	0.482	0.716		
A96-6/DLB (no AD)	No AD #5	5.520	0.227	4.423	0.240	1.097	0.467		
A96-12/DLB (no AD)	No AD #6	4.876	0.116	4.660	0.215	0.216	0.861		
A95-48/DLB (no AD)	No AD #7	5.168	0.174	4.619	0.057	0.548	0.684		
Temporal Parietal Cortex: Mean | SD		5.067	0.280						
Cerebellum: Mean | SD				4.485	0.286				

Table 9

Relative APCL mRNA Levels in Human Brain ["delta"Ct Value (APCL – Actin)] in Human Brain Samples from AD and Other Neurological Diseases

Pathologist's Diagnosis	Patient ID#	ΔCt TPC (Temporal Parietal Cortex) Mean	SD	ΔCt Cere (Cerebellum) Mean	SD	$\Delta\Delta$Ct = ΔCt TPC — ΔCt Cere	$2^{-\Delta\Delta Ct}$ = Fold Change Per Patient	*Fold Change* in Patient Sub groups Mean	SD
A98-19,11 (Control)	Control	10.593	0.232	9.170	0.230	1.423	0.373		
A98-01/sAD (Braak stage VI)	sAD #1	11.212	0.314	10.510	0.108	0.702	0.615		
A98-07/AD (Braak stage VI and DLB)	sAD #2	10.934	0.172	10.664	0.140	0.270	0.829		
A97-7/sAD (Braak stage VI)	sAD #3	11.085	0.014	9.766	0.010	1.319	0.401	0.660	0.189
A97-9/sAD (Braak stage VI and DLB)	sAD #4	10.473	0.026	9.728	0.361	0.745	0.597		
A96-8/sAD (Braak stage VI)	sAD #5	10.963	0.092	10.746	0.109	0.217	0.861		
Temporal Parietal Cortex: Mean | SD		10.933	0.280						
Cerebellum: Mean | SD				10.283	0.497				
A98-18/AD (V)	mAD #1	9.514	0.360	9.178	0.321	0.337	0.792		
A96-25/AD (IV)	mAD #2	11.213	0.077	9.287	0.239	1.927	0.263		
A97-05/mAD (V)	mAD #3	10.053	0.187	9.168	0.204	0.884	0.542	0.534	0.213
A97-10/sAD (V and DLB)	mAD #4	10.043	0.142	8.386	0.282	1.658	0.317		
A96-23/AD III and VaD and DLB	mAD #5	8.773	0.338	8.328	0.221	0.446	0.734		
A98-04/limbic AD III	mAD #6	11.143	0.259	10.292	0.204	0.851	0.554		
Temporal Parietal Cortex: Mean | SD		10.123	0.941						
Cerebellum: Mean | SD				9.106	0.717				
A96-11/VaD (no AD change)	No AD#1	11.459	0.187	9.602	0.337	1.858	0.276		
A97-11/mixed (corticobasal degeneration &	No	9.025	0.365	8.436	0.333	0.589	0.665		

Pathologist's Diagnosis	Patient ID#	ΔCt TPC (Temporal Parietal Cortex) Mean	SD	ΔCt Cere (Cerebellum) Mean	SD	ΔΔCt = ΔCt TPC — ΔCt Cere	$2^{-\Delta\Delta Ct}$ = Fold Change Per Patient	*Fold Change* in Patient Sub groups Mean	SD
PD)	AD#2								
A97-18/VaD (no AD changes)	No AD#3	9.528	0.232	8.282	0.128	1.245	0.422		
A97-19/VaD (no AD changes)	No AD#4	11.500	0.277	10.963	0.434	0.538	0.689	0.387	0.212
A96-6/DLB (no AD)	No AD#5	11.128	0.161	8.571	0.246	2.557	0.170		
A96-12/DLB (no AD)	No AD#6	9.155	0.446	7.186	0.279	1.969	0.255		
A95-48/DLB (no AD)	No AD#7	11.297	0.541	9.197	0.268	2.100	0.233		
Temporal Parietal Cortex: Mean | SD		10.442	1.144						
Cerebellum: Mean | SD				8.891	1.189				

Table 7, as well as other Tables 6, 8 and 9, evaluates data from above experiments in a variety of ways. In order to understand the various results from the data analysis, it must first be recognized that a lower "delta" Ct value corresponds to higher levels of the mRNA in question. The "delta" Ct values from two regions of the brain thought to be affected in AD are compared in several ways. These analyses of the data use some of the mathematical formulae presented in preceding examples.

To begin, the ratio "$2^{-\Delta\Delta \text{ Ct}}$" represents the difference in expression of FREAC-2 in a patient's TPC as compared to expression in the cerebellum (*e,g.,* lines 2-6, second to rightmost column. The rightmost column of the table shows the mean (as well as the standard deviation, SD) of the ratio "$2^{-\Delta\Delta \text{ Ct}}$" from three subgroups of patients. These values are 3.5, 1.8 and 1.6, respectively, for patients having severe AD (sAD), moderate AD (mAD) and patients not having AD (controls).

Because the results described above are ratios, they can be achieved in two different ways. A ratio of 3.5 for the relative levels of FREAC-2 mRNA in the TPC as compared to the cerebellum indicates that "more" FREAC-2 mRNA is present in the TPC. This can be realized in 2 different ways: FREAC-2 might be down-regulated in the cerebellum in AD, and/or FREAC-2 might be up-regulated in the TPC. Comparison of other values in Table D2 to one another helps distinguish these causes for the differential expression of FREAC-2 in AD brains.

For example, the mean "delta" Ct for TPC FREAC-2 in each patient subgroup is determined; the values for sAD, mAD and no AD are, respectively, 9.9, 11.2 and 11.6 (see lines 7, 15 and 24 of Table D2). Because a lower "delta" Ct TPC corresponds to a higher level of FREAC-2 mRNA, the value of 9.9 for sAD, as compared to 11.2 and 11.6 for mAD and no AD, indicates that FREAC-2 mRNA is present in greater amounts in AD TPCs, i.e., FREAC-2 is up-regulated in AD TPCs.

Similarly, the mean "delta" Ct for Cerebellum FREAC-2 in each patient subgroup is determined; the values for sAD, mAD and no AD are, respectively, 11.5, 11.9 and 12.1 (see lines 8, 16 and 25 of Table D2). Because a lower "delta" Ct TPC corresponds to a higher level of FREAC-2 mRNA, the value of 11.5 for sAD, as compared to 11.9 and 12.1 for mAD and no AD, indicates that FREAC-2 mRNA is

present in greater amounts in cerebellums from sAD patients, i.e., FREAC-2 is up-regulated in AD cerebellums. However, the differences between the mean "delta" Ct's for Cerebellum FREAC-2 are less pronounced than those for the TPC, suggesting that differences in the amounts of FREAC-2 in the various cerebellums is slight. Moreover, an increased level of FREAC-2 in cerebellum requires higher than normal levels of FREAC-2 in the TPC in order to achieve a ratio that indicates "more" FREAC-2 is present in the TPC. Taking all of the data into account, the results suggest that FREAC-2 is up-regulated in the TPC of sAD patients.

All publications, including patent documents and scientific articles, referred to in this application are incorporated by reference in their entirety for all purposes to the same extent as if each individual publication were individually incorporated by reference.

From the foregoing, it will be appreciated that, although specific embodiments of the invention have been described herein for purposes of illustration, various modifications may be made without deviating from the spirit and scope of the invention. Accordingly, the invention is not limited except as by the appended claims.

CLAIMS

What is claimed is:

1. An oligonucleotide primer capable of specifically amplifying DNA or RNA encoding contactin or a nucleic acid sequence complementary thereto, comprising an isolated nucleic acid molecule that is identical or substantially identical to a nucleotide sequence selected from the group consisting of SEQ ID NO:1 or a portion thereof, SEQ ID NO:2 or a portion thereof, SEQ ID NO:3 or a portion thereof, SEQ ID NO:4 or a portion thereof, SEQ ID NO:5 or a portion thereof, SEQ ID NO:6 or a portion thereof and SEQ ID NO:7 or a portion thereof.

2. The composition of claim 1, wherein said isolated nucleic acid molecule comprises a detectable label.

3. A kit, comprising: at least one oligonucleotide primer capable of specifically amplifying DNA or RNA encoding contactin or a nucleic acid sequence complementary thereto, comprising an isolated nucleic acid molecule that is identical or substantially identical to a nucleotide sequence selected from the group consisting of SEQ ID NO:1 or a portion thereof, SEQ ID NO:2 or a portion thereof, SEQ ID NO:3 or a portion thereof, SEQ ID NO:4 or a portion thereof, SEQ ID NO:5 or a portion thereof, SEQ ID NO:6 or a portion thereof and SEQ ID NO:7 or a portion thereof.

4. The kit of claim 3, wherein said at least one nucleic acid molecule comprises a detectable label.

5. A method of detecting the risk for having or presence of a neurodegenerative disease in a subject, comprising:

comparing the amount of contactin protein in a test sample from a subject to the amount of contactin protein in a sample from a control subject known to be free of a risk for having or presence of the neurodegenerative disease.

6. A method of detecting the risk for having or presence of a neurodegenerative disease in a subject, comprising:

comparing the amount of contactin mRNA in a test sample from a subject to the amount of contactin mRNA in a sample from a control subject known to be free of a risk for having or presence of the neurodegenerative disease.

7. The method of either claim 5 or 6 wherein said test sample and said control sample are derived from central nervous system.

8. The method of claim 6 wherein said contactin mRNA is measured by a polymerase chain reaction method.

9. The method of claim 8, wherein said polymerase chain reaction method comprises amplification with a forward primer comprising a nucleotide sequence selected from the group consisting of SEQ ID NO:1, SEQ ID NO:3, SEQ. ID NO:5 and SEQ ID NO:6.

10. The method of claim 8, wherein said polymerase chain reaction method comprises amplification with a reverse primer comprising a nucleotide sequence selected from the group consisting of SEQ ID NO:2, SEQ ID NO:4, and SEQ ID NO: 7.

11. A method of detecting the risk for having or presence of a neurodegenerative disease in a subject, comprising:

comparing the amount of contactin mRNA in a test sample from a subject to the amount of RNA in a sample from a control subject known to be free of a risk for having

or presence of the neurodegenerative disease, wherein said control sample RNA does not encode contactin.

12. The method of either claim 5 or 6 wherein the neurodegenerative disease is selected from the group consisting of amyotrophic lateral sclerosis, multiple sclerosis, MELAS and MERRF.

13. The method of either claim 5 or 6 wherein the neurodegenerative disease is multiple sclerosis.

14. A method of screening an agent for use in treating patients having a neurodegenerative disease, comprising:

determining a first level of contactin expression in a first sample from at least one patient prior to contacting the patient with a candidate agent, wherein the step of determining is selected from the group consisting of determining an amount of contactin protein and determining an amount of contactin RNA; and

comparing said first level of contactin expression to a second level of contactin expression determined in a second sample from the patient after contacting the patient with the candidate agent, wherein a change in the level of contactin expression indicates that the agent is suitable for use in treating patients having a neurodegenerative disease.

15. A cybrid cell line comprising immortal and differentiatable cells having genomic and mitochondrial DNA of differing biological origins, said cells expressing contactin.

16. The cybrid cell line of claim 15 wherein the cells are neural cells.

17. The cybrid cell line of claim 15 wherein the cells are human cells.

18. The cybrid cell line of claim 15 wherein the cells are human central nervous system cells.

19. A method of identifying an agent capable of altering contactin expression in a cell comprising:

comparing the level of contactin expression in at least one cell before and after contacting said at least one cell with a candidate agent, and therefrom identifying an agent capable of altering contactin expression.

20. The method of claim 19, wherein the candidate agent comprises a test compound selected from the group consisting of a small molecule, a nucleic acid molecule, an antisense nucleic acid molecule and a ribozyme.

21. The method of claim 19, wherein said at least one cell comprises a cybrid cell.

22. An agent capable of altering contactin expression identified by the method of claim 19.

23. The agent of claim 22 that is selected from the group consisting of a small molecule, a protein, a polypeptide, an antibody, a nucleic acid molecule, an antisense molecule and a ribozyme.

24. A pharmaceutical composition comprising the agent of claim 22 in a pharmaceutically acceptable carrier.

25. A method of treating a patient having a neurodegenerative disease, comprising administering to the patient an effective amount of the pharmaceutical composition of claim 24.

26. A method of treating a patient having a neurodegenerative disease, comprising:

administering to said patient an effective amount of a pharmaceutical composition capable of altering contactin expression.

27. A pharmaceutical target comprising a cellular component that binds to the agent of claim 22.

28. A method of identifying a pharmaceutical target, comprising contacting a compound that modulates contactin expression or activity with a biological sample known to exhibit contactin expression or activity.

29. A method for determining the risk for or presence of Alzheimer's disease in a first subject suspected of having or being at risk for having such a disease, comprising:

determining the presence or absence of at least one differentially expressed nucleic acid molecule that is associated with Alzheimer's disease in each of a first and a second biological sample comprising mitochondrial DNA, said first biological sample being obtained from said first subject and said second sample being obtained from a second subject known to be free of a risk or presence of a disease associated with altered mitochondrial function,

wherein the presence of at least one differentially expressed nucleic acid molecule that is associated with Alzheimer's disease in said first biological sample and the absence of a corresponding differentially expressed nucleic acid molecule having a corresponding nucleotide sequence in said second biological sample indicates an increased risk of Alzheimer's disease,

and therefrom determining the risk or presence of Alzheimer's disease.

30. The method of claim 29 wherein the step of determining comprises contacting each of said first and second biological samples with a nucleic acid array comprising a plurality of isolated nucleic acid molecules immobilized on a solid support, wherein said isolated nucleic acid molecules comprise at least one differentially expressed nucleic acid molecule that is associated with Alzheimer's disease, under conditions and for a time sufficient to allow hybridization of DNA from said samples to said isolated nucleic acid molecules; and

comparing an amount of hybridization to the nucleic acid array of (i) the nucleic acid molecule that is differentially expressed and that is associated with Alzheimer's disease of the first sample, to (ii) an amount of hybridization of the nucleic acid of the second sample that corresponds to the nucleic acid molecule that is differentially expressed and that is associated with Alzheimer's disease in the first sample, and therefrom determining the presence or absence of at least one differentially expressed nucleic acid molecule that is associated with Alzheimer's disease.

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
23 November 2000 (23.11.2000)

PCT

(10) International Publication Number
WO 00/70099 A3

(51) International Patent Classification[7]: C12Q 1/68, C12N 5/10

(21) International Application Number: PCT/US00/13951

(22) International Filing Date: 19 May 2000 (19.05.2000)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
60/134,790 19 May 1999 (19.05.1999) US
09/574,613 18 May 2000 (18.05.2000) US

(71) Applicant (*for all designated States except US*): **MITOKOR** [US/US]; 11494 Sorrento Valley Road, San Diego, CA 92121 (US).

(72) Inventors; and

(75) Inventors/Applicants (*for US only*): **HWANG, Jung-Joo** [KR/US]; #4, 3773 Carmel View Road, San Diego, CA 92130 (US). **BECKER, K., David** [US/US]; 12056 Mannix Road, San Diego, CA 92129 (US). **DAVIS, Robert, E.** [US/US]; 13272 Glencliff Way, San Diego, CA 92130 (US).

(74) Agents: **ROSENMAN, Stephen, J.** et al.; Seed Intellectual Property Law Group PLLC, Suite 6300, 701 Fifth Avenue, Seattle, WA 98104-7092 (US).

(81) Designated States (*national*): AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW.

(84) Designated States (*regional*): ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).

Published:
— *with international search report*

(88) Date of publication of the international search report: 4 April 2002

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

(54) Title: DIFFERENTIAL GENE EXPRESSION IN SPECIFIC REGIONS OF THE BRAIN IN NEURODEGENERATIVE DISEASES

WO 00/70099 A3

(57) Abstract: The present invention provides nucleic acid molecules that can function as PCR primers for the detection of contactin mRNA in a sample. Such nucleic acid molecules can be labeled and can be provided in a kit. The present invention also includes a method of detecting the presence of a neurodegenerative disease such as multiple sclerosis, including providing a sample from a patient and measuring the amount of contactin protein or contactin mRNA expressed in the sample. The present invention further provides a method for identifying which patents having a neurodegenerative disease are likely to respond to a treatment for a neurodegenerative disease. The present invention further includes compositions of matter that include an isolated cell or a cell in culture that expresses an increased or decreased amount of contactin as compared to a control cell. The present invention also includes a method for screening compounds for the activity of reducing or enhancing the expression of contactin and compositions or compounds, including pharmaceutical compositions, identified by this method. Also included are methods of treating a neurodegenerative disease using such compositions or compounds. The present invention also includes methods of identifying pharmaceutical targets for compounds that modulate contactin expression or activity and targets identified by such methods.

INTERNATIONAL SEARCH REPORT

International Application No
PCT/US 00/13951

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 C12Q1/68 C12N5/10

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 C12Q C12N G01N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

EPO-Internal, SEQUENCE SEARCH

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 5 739 289 A (HEMPERLY JOHN JACOB ET AL) 14 April 1998 (1998-04-14) column 4, line 55 -column 5, line 33 ---	1-4
X	PLAGGE A ET AL: "The gene of the neural cell recognition molecule F11: conserved exon-intron arrangement in genes of neural members of the immunoglobulin superfamily" GENE: AN INTERNATIONAL JOURNAL ON GENES AND GENOMES, ELSEVIER SCIENCE PUBLISHERS, BARKING, GB, vol. 192, no. 2, 1997, pages 215-225, XP004081714 ISSN: 0378-1119 page 216, paragraph 3 -page 217, paragraph 1 --- -/--	1-4

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
20 September 2001	21 12 2001

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	OSBORNE, H

2

INTERNATIONAL SEARCH REPORT

International Application No
PCT/US 00/13951

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 95 35373 A (JOLLA CANCER RES FOUND) 28 December 1995 (1995-12-28) page 22, line 25 -page 23, line 4 ---	1-4
A	SHIMAZAKI K ET AL: "Age-related decline of f3/contactin in rat hippocampus" NEUROSCIENCE LETTERS, vol. 245, no. 2, 1998, pages 117-20, XP001024403 ---	
A	US 5 888 498 A (DAVIS ROBERT E ET AL) 30 March 1999 (1999-03-30) the whole document ---	15-18
A	WO 97 15690 A (CURAGEN CORP) 1 May 1997 (1997-05-01) the whole document ---	5-14
A	WO 98 49342 A (COCKBAIN JULIAN R M ;FORSKNINGSPARKEN I AAS AS (NO); LOENNEBORG AN) 5 November 1998 (1998-11-05) see whole document, especially pages 2-6 -----	5-14

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 00/13951

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

see additional sheet

1. ☐ As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

1-28

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest.

☐ No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

This International Searching Authority found multiple (groups of) inventions in this international application, as follows:

1. Claims: 1-28

   Contactin specific primers and uses thereof in methods for diagnosing the risk of having a neurodegenerative disease and in methods for screening for therapeutically effective agents; and cell lines expressing contactin.

2. Claims: 29,30

   A method for assessing the risk for the presence of Alzheimer's disease in a subject by monitoring the differential expression of mitochondrial DNA.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No
PCT/US 00/13951

Patent document cited in search report		Publication date	Patent family member(s)			Publication date
US 5739289	A	14-04-1998	US	5688916	A	18-11-1997
			US	6017695	A	25-01-2000
			AU	685417	B2	22-01-1998
			AU	5771894	A	29-09-1994
			CA	2119974	A1	27-09-1994
			EP	0618293	A1	05-10-1994
			JP	2690266	B2	10-12-1997
			JP	6319555	A	22-11-1994
			US	5731154	A	24-03-1998
WO 9535373	A	28-12-1995	AU	2824195	A	15-01-1996
			WO	9535373	A2	28-12-1995
US 5888498	A	30-03-1999	US	5565323	A	15-10-1996
			AU	705230	B2	20-05-1999
			AU	2204295	A	23-10-1995
			BR	9507241	A	16-09-1997
			CA	2186636	A1	12-10-1995
			CN	1150433	A	21-05-1997
			EP	0751951	A1	08-01-1997
			FI	963884	A	26-11-1996
			JP	9511398	T	18-11-1997
			NO	964073	A	29-11-1996
			NZ	283660	A	28-07-1998
			WO	9526973	A1	12-10-1995
			US	6146831	A	14-11-2000
			US	6291172	B1	18-09-2001
			US	5840493	A	24-11-1998
			US	6027883	A	22-02-2000
			US	2001021526	A1	13-09-2001
			US	6171859	B1	09-01-2001
			US	5760205	A	02-06-1998
			US	5976798	A	02-11-1999
WO 9715690	A	01-05-1997	US	5871697	A	16-02-1999
			US	5972693	A	26-10-1999
			AU	730830	B2	15-03-2001
			AU	7476396	A	15-05-1997
			EP	0866877	A1	30-09-1998
			IL	124185	A	06-12-2000
			JP	2000500647	T	25-01-2000
			WO	9715690	A1	01-05-1997
			US	6231812	B1	15-05-2001
			US	2001007985	A1	12-07-2001
			US	6141657	A	31-10-2000
WO 9849342	A	05-11-1998	AU	7222698	A	24-11-1998
			EP	0979308	A1	16-02-2000
			WO	9849342	A1	05-11-1998
			NO	995296	A	14-12-1999